

U.S. ENVIRONMENTAL PROTECTION AGENCY

AGENCY USE ONLY

PREMANUFACTURE
NOTICE

When
completed
send this
form to

Date of receipt

09 SEP 16 AM 11:31

Company Sanitized

Enter the total number of pages
in the Premanufacture Notice

Document control number

51090000631

EPA case number

P09-631

GENERAL INSTRUCTIONS

TS - C 0 0 1 H E

- You must provide all information requested in this form to the extent that it is known to or reasonably ascertainable by you. Make reasonable estimates if you do not have actual data.
- Before you complete this form, you should read the "Instructions Manual for Premanufacture Notification" (the Instructions Manual is available from the Toxic Substances Control Act (TSCA) Information Service by calling 202-554-1404, or faxing 202-554-5603).
- If a user fee has been remitted for this notice (40 CFR 700.45), indicate in the boxes above the TS-user fee identification number you have generated. Remember, your user fee ID number must also appear on your corresponding fee remittance, which is sent to EPA, HQ Accounting Operations Branch (PM-226), P.O. 360399M, Pittsburgh, PA 15251-6399, Attn. TSCA User fee.

Part I—GENERAL INFORMATION

You must provide the currently correct Chemical Abstracts (CA) Name of the new chemical substance, even if you claim the identity as confidential. You may authorize another person to submit chemical identity information for you, but your submission will not be complete and the review will not begin until EPA receives this information. A letter in support of your submission should reference your TS user fee identification number. You must submit an original and two copies of this notice including all test data. If you claimed any information as confidential, a single sanitized copy must also be submitted.

Part II—HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE

If there are several manufacture, processing, or use operations to be described in Part II, sections A and B of this notice, reproduce the sections as needed.

Part III—LIST OF ATTACHMENTS

Attach additional sheets if there is not enough space to answer a question fully. Label each continuation sheet with the corresponding section heading. In Part III, list these attachments, any test data or other data and any optional information included in the notice.

OPTIONAL INFORMATION

You may include any information that you want EPA to consider in evaluating the new substance. On page 11 of this form, space has been provided for you to described pollution prevention and recycling information you may have regarding the new substance.

So-called "binding" boxes are included throughout this form for you to indicate your willingness to be bound to certain statements you make in this section, such as use, production volume, protective equipment . . . This option is intended to reduce delays that routinely accompany the development of consent orders or Significant New Use Rules. Except in the case of exemption applications (such as TMEA, LVE, LOREX) where certain information provided in such notification is binding on the submitter when the Agency approves the exemption application, checking a binding box in this notice does not by itself prohibit the submitter from later deviating from the information (except chemical identity) reported in the form.

CONFIDENTIALITY CLAIMS

You may claim any information in this notice as confidential. To assert a claim on the form, mark (X) the confidential box next to the information that you claim as confidential. To assert a claim in an attachment, circle or bracket the information you claim as confidential. If you claim information in the notices as confidential, you must also provide a sanitized version of the notice, (including attachments). For additional instructions on claiming information as confidential, read the Instructions Manual.



Mark (x) if any information in this notice is claimed as confidential.

TEST DATA AND OTHER DATA

You are required to submit all test data in your possession or control and to provide a description of all other data known to or reasonably ascertainable by you, if these data are related to the health and environmental effects on the manufacture, processing, distribution in commerce, use, or disposal of the new chemical substance. Standard literature citations may be submitted for data in the open scientific literature. Complete test data (written in English), not summaries of data, must be submitted if they do not appear in the open literature. You should clearly identify whether test data is on the substance or on an analog. Also, the chemical composition of the tested material should be characterized. Following are examples of test data and other data. Data should be submitted according to the requirements of §720.50 of the Premanufacture Notification Rule (40 CFR Part 720).

Test Data (Check Below any included in this notice)

- | | | | |
|---------------------------------|---|---|------------------------------|
| • Environmental fate data | <input type="checkbox"/> Yes | • Other data | <input type="checkbox"/> Yes |
| • Health effects data | <input checked="" type="checkbox"/> Yes | Risk assessments | |
| • Environmental effects data | <input checked="" type="checkbox"/> Yes | Structure/activity relationships | |
| • Physical/Chemical Properties* | <input checked="" type="checkbox"/> Yes | Test data not in the possession or control of the submitter | |

* A physical and chemical properties worksheet is located on the last page of this form.

TYPE OF NOTICE

(Check Only One)

- | | |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | PMN (Premanufacture Notice) |
| <input type="checkbox"/> | INTERMEDIATE PMN (submitted in sequence with final product PMN) |
| <input type="checkbox"/> | SNUN (Significant New Use Notice) |
| <input type="checkbox"/> | TMEA (Test Marketing Exemption Application) |
| <input type="checkbox"/> | LVE (Low Volume Exemption) @ 40 CFR 723.50(c)(1) |
| <input type="checkbox"/> | LOREX (Low Release/Low Exposure Exemption) @ 40 CFR 723.50(c)(2) |
| <input type="checkbox"/> | LVE Modification |
| <input type="checkbox"/> | LOREX Modification |

IS THIS A CONSOLIDATED PMN?



Yes

of chemicals

(Prenotice Communication # required, enter # on page 3)

Sanitized
COPY

MR 321567

Public reporting burden for this collection of information is estimated to average 110 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M. St., S.W., Washington, D.C. 20460; and to the Office of Management and Budget, Paperwork Reduction Act (2070-0012), Washington, D.C. 20503.

CERTIFICATION

I certify that to the best of my knowledge and belief:

1. The company named in Part I, section A, subsection 1a of this notice form intends to manufacture or import for a commercial purpose, other than in small quantities solely for research and development, the substance identified in Part I, Section B.
2. All information provided in this notice is complete and truthful as of the date of submission.
3. I am submitting with this notice all test data in my possession or control and a description of all other data known to or reasonably ascertainable by me as required by §720.50 of the Premanufacture Notification Rule.

Additional Certification Statements:

If you are submitting a PMN, Intermediate PMN, Consolidated PMN, or SNUN, check the following **user fee** certification statement that applies:

- ☒ The Company named in Part I, Section A has remitted the fee of \$2500 specified in 40 CFR 700.45(b), or
- ☐ The Company named in Part I, Section A has remitted the fee of \$1000 for an Intermediate PMN (defined @ 40 CFR 700.43) in accordance with 40 CFR 700.45(b), or
- ☐ The Company named in Part I Section A is a small business concern under 40 CFR 700.43 and has remitted a fee of \$100 in accordance with 40 CFR 700.45(b).

If you are submitting a **low volume exemption (LVE)** application in accordance with 40 CFR 723.50(c)(1) or a **Low release and low exposure exemption (LoRex)** application in accordance with 40 CFR 723.50(c)(2), check the following certification statements:

- ☐ The manufacturer submitting this notice intends to manufacture or import the new chemical substance for commercial purposes, other than in small quantities solely for research and development, under the terms of 40 CFR 723.50.
- ☐ The manufacturer is familiar with the terms of this section and will comply with those terms; and
- ☐ The new chemical substance for which the notice is submitted meets all applicable exemption conditions.
- ☐ If this application is for an LVE in accordance with 40 CFR 723.50(c)(1), the manufacturer intends to commence manufacture of the exempted substance for commercial purposes within 1 year of the date of the expiration of the 30 day review period.

The accuracy of the statements you make in this notice should reflect your best prediction of the anticipated facts regarding the chemical substance described herein. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 USC 1001.

Confidential

Signature and title of Authorized Official (Original Signature Required) <div style="border: 1px solid black; width: 100%; height: 40px; margin-top: 5px;"></div>	Date <div style="border: 1px solid black; width: 100%; height: 40px; margin-top: 5px;"></div>	Confidential X
Signature of agent - (if applicable)	Date	

Part I -- GENERAL INFORMATION

Section A -- SUBMITTER IDENTIFICATION

Mark () the "Confidential" box next to any subsection you claim as confidential

Confidential

1a. Person Submitting Notice (in U.S.)	Name of authorized official []	Position []			X
	Company []				
	Mailing address (number and street) []				
	City, State, ZIP Code []				
b. Agent (if applicable)	Name of authorized official	Position			
	Company N/A				
	Mailing address (number and street)				
	City, State, ZIP Code	Telephone	Area Code	Number	
c. If you are submitting this notice as part of a joint submission, mark (X) this box. <input type="checkbox"/>					
Joint Submitter (if applicable)	Name of authorized official N/A	Position			
	Company				
	Mailing address (number and street)				
	City, State, ZIP Code	Telephone	Area Code	Number	
2. Technical Contact (in U.S.)	Name of authorized official []	Position []			X
	Company []				
	Mailing address (number and street) []				
	City, State, ZIP Code	Telephone	Area Code	Number	
	[]	[]		[]	
3. If you have had a prenotice communication (PC) concerning this notice and EPA assigned a PC Number to the notice, enter the number. →			Mark (X) if none →	<input checked="" type="checkbox"/>	
4. If you previously submitted an exemption application for the chemical substance covered by this notice, enter the exemption number assigned by EPA. If you previously submitted a PMN for this substance enter the PMN number assigned by EPA (i.e. withdrawn or incomplete). →			Mark (X) if none →	<input checked="" type="checkbox"/>	
5. If you have submitted a notice of Bona fide intent to manufacture or import for the chemical substance covered by this notice, enter the notice number assigned by EPA. →			Mark (X) if none →	<input checked="" type="checkbox"/>	
6. Type of Notice - Mark (X)	1. <input type="checkbox"/> Manufacture Only <input type="checkbox"/> Binding Option Mark (X)	2. <input checked="" type="checkbox"/> Import Only <input type="checkbox"/> Binding Option Mark (X)	3. <input type="checkbox"/> Both		

Part I -- GENERAL INFORMATION -- Continued**Section B -- CHEMICAL IDENTITY INFORMATION:**

You must provide a currently correct Chemical Abstracts (CA) name of the substance based on the ninth Collective Index (9CI) of CA nomenclature rules and conventions.

Mark (X) the "Confidential" box next to any item you claim as confidential

Complete either item 1 (Class 1 or 2 substances) or 2 (Polymers) as appropriate. Complete all other items.

If another person will submit chemical identity information for you (for either item 1 or 2), mark (X) the box at the right. Identify the name, company, and address of that person in a continuation sheet.



Confidential

1. Class 1 or 2 chemical substances (for definitions of class 1 and class 2 substances, see the Instructions Manual)

a. Class of substance - Mark (X) 1 ☐ Class 1 or 2 ☒ Class 2

b. Chemical name (Currently correct Chemical Abstracts (CA) Name that is consistent with TSCA Inventory listings for similar substances. For Class 1 substances a CA Index Name must be provided. For Class 2 substances either a CA Index Name or CA Preferred Name must be provided, which ever is appropriate based on CA 9CI nomenclature rules and conventions).

X

[]

c. Please identify which method you used to develop or obtain the specified chemical identity information reported in this notice: (check one).



Method 1 (CAS Inventory Expert Service - a copy of the Identification report obtained from the CAS Inventory Expert Services must be submitted as an attachment to this notice)



Method 2 (Other Source)

d. Molecular formula and CAS Registry Number (if a number already exists for the substance)

[]

X

CAS#

[]

X

e. For a class 1 substance, provide a complete and correct chemical structure diagram. For a class 2 substance - (1) List the immediate precursor substances with their respective CAS Registry Numbers. (2) Describe the nature of the reaction or process. (3) Indicate the range of composition and the typical composition (where appropriate). (4) Provide a correct representative or partial chemical structure diagram, as complete as can be known, if one can be reasonably ascertained.

X

[]

See Page 14 Continuation Sheet



Mark (X) this box if you attach a continuation sheet

Part I -- GENERAL INFORMATION -- Continued

Section B -- CHEMICAL IDENTITY INFORMATION -- Continued

2. Polymers (For a definition of polymer, see the Instructions Manual.)

Confidential

- a. Indicate the number-average weight of the lowest molecular weight composition of the polymer you intend to manufacture. Indicate maximum weight percent of low molecular weight species (not including residual monomers, reactants, or solvents) below 500 and below 1,000 absolute molecular weight of that composition.

Describe the methods of measurement or the basis for your estimates: GPC ☐ Other ☐: (Specify) _____

i) lowest number average molecular weight: _____

ii) maximum weight % below 500 molecular weight: N/A

iii) maximum weight % below 1000 molecular weight: _____

☐ Mark (X) this box if you attach a continuation sheet.

- b. You must make separate confidentiality claims for monomer or other reactant identity, composition information, and residual information. Mark (X) the "Confidential" box next to any item you claim as confidential

- (1) - Provide the specific chemical name and CAS Registry Number (if a number exists) of each monomer or other reactant used in the manufacture of the polymer.
 (2) - Mark (X) this column if entry in column (1) is confidential.
 (3) - Indicate the typical weight percent of each monomer or other reactant in the polymer.
 (4) - Mark (X) the identity column if you want a monomer or other reactant used at two weight percent or less to be listed as part of the polymer description on the TSCA Chemical Substance Inventory.
 (5) - Mark (X) this column if entries in columns (3) and (4) are confidential.
 (6) - Indicate the maximum weight percent of each monomer or other reactant that may be present as a residual in the polymer as manufactured for commercial purposes.
 (7) - Mark (X) this column if entry in column (6) is confidential.

Monomer or other reactant and CAS Registry Number (1)	Confidential (2)	Typical composition (3)	Identity Mark (X) (4)	Confidential (5)	Maximum residual (6)	Confidential (7)
		%			%	
		%			%	
		%			%	
Not Applicable		%			%	
		%			%	
		%			%	
		%			%	

☐ Mark (X) this box if you attach a continuation sheet.

- c. Please identify which method you used to develop or obtain the specified chemical identity information reported in this notice (check one).

☐ Method 1 (CAS Inventory Expert Service - a copy of the identification report ☐ Method 2 (other source)

obtained from CAS Inventory Expert Service must be submitted as
as attachment to this notice)

- d. The currently correct Chemical Abstracts (CA) name for the polymer that is consistent with TSCA Inventory listings for similar polymers.

- e. Provide a correct representative or partial chemical structure diagram, as complete as can be known, if one can be reasonably ascertained.

Not applicable

☐ Mark (X) this box if you attach a continuation sheet.

Part I -- GENERAL INFORMATION -- Continued

Section B -- CHEMICAL IDENTITY INFORMATION -- Continued

3. Impurities

- (a) - Identify each impurity that may be reasonably anticipated to be present in the chemical substance as manufactured for commercial purpose. Provide the CAS Registry Number if available. If there are unidentified impurities, enter "unidentified."
- (b) - Estimate the maximum weight % of each impurity. If there are unidentified impurities, estimate their total weight %.

Impurity and CAS Registry Number (a)	Maximum percent (b)	Confidential
[]	[]	X

☐ Mark (X) this box if you attach a continuation sheet.

4. Synonyms - Enter any chemical synonyms for the new chemical identified in subsection 1 or 2.

N/A

Confidential

☐ Mark (X) this box if you attach a continuation sheet.

5. Trade identification - List trade names for the new chemical substance identified in subsection 1 or 2.

[]

☐ Mark (X) this box if you attach a continuation sheet.

6. Generic chemical name - If you claim chemical identity as confidential, you must provide a generic name for your substance that reveals the specific chemical identity of the new chemical substance to the maximum extent possible. Refer to the TSCA Chemical Substance Inventory, 1985 Edition, Appendix B for guidance on developing generic names.

Bromodiphenylethane

☐ Mark (X) this box if you attach a continuation sheet.

7. Byproducts - Describe any byproducts resulting from the manufacture, processing, use, or disposal of the new chemical substance. Provide the CAS Registry Number if available.

Byproduct (1)	CAS Registry Number (2)	Confidential
N/A		

☐ Mark (X) this box if you attach a continuation sheet.

Part I -- GENERAL INFORMATION -- Continued**Section C -- PRODUCTION, IMPORT, AND USE INFORMATION:**

Mark (X) the "Confidential" box next to any item you claim as confidential.

- 1. Production volume** -- Estimate the **maximum** production volume during the first 12 months of production. Also estimate the maximum production volume for any consecutive 12-month period during the first three years of production. Estimates should be on 100% new chemical substance basis. For a Low Volume Exemption application, if you choose to have your notice reviewed at a lower production volume than 10,000 kg/yr, specify the volume and mark (x) in the binding box. If granted, you are bound to this volume

Maximum first 12-month production (kg/yr) (100% new chemical substance basis)	Maximum 12-month production (kg/yr) (100% new chemical substance basis)	Confidential	Binding Option Mark (x)
[]	[]	X	

- 2. Use Information** -- You must make separate confidentiality claims for the description of the category of use, the percent of production volume devoted to each category, the formulation of the new substance, and other use information. Mark (X) the "Confidential" Box next to any item you claim as confidential.
- a. (1) -- Describe each intended category of use of the new chemical substance by function and application..
- (2) -- Mark (X) this column if entry column (1) is confidential business information (CBI).
- (3) -- Indicate your willingness to have the information provided in column (1) binding.
- (4) -- Estimate the percent of total production for the first three years devoted to each category of use.
- (5) -- Mark (X) this column if entry in column (4) is confidential business information (CBI).
- (6) -- Estimate the percent of the new substance as formulated in mixtures, suspensions, emulsions, solutions, or gels as manufactured for commercial purposes at sites under your control associated with each category of use.
- (7) -- Mark (X) this column if entry in column (6) is confidential business information (CBI).
- (8) -- Indicate % of product volume expected for the listed "use" sectors. Mark more than one box if appropriate. Mark (X) to indicate your willingness to have the use type provided in (8) binding.
- (9) -- Mark (X) this column if entry(ies) in column (8) is (are) confidential business information (CBI).

Category of use (1) (by function and application i.e. a dispersive dye for finishing polyester fibers)	CBI (2)	Binding Option Mark (x) (3)	Production % (4)	CBI (5)	% in Formulation (6)	CBI (7)	% of substance expected per use (8)					CBI (9)
							Site-limited	Con-*sumer	Indus-trial	Com-mercial	Binding Option	
Flame Retardant for molded electrical devices			[]	X	[]	X			[]			X
			%		%							
			%		%							
			%		%							
			%		%							
			%		%							
			%		%							
			%		%							

* If you have identified a "consumer" use, please provide on a continuation sheet a detailed description of the use(s) of this chemical substance in consumer products. In addition include estimates of the concentration of the new chemical substance as expected in consumer products and describe the chemical reactions by which this substance loses its identity in the consumer product.

☐ Mark (X) this box if you attach a continuation sheet.

b. Generic use description	If you claim any category of use description in subsection 2a as confidential, enter a generic description of that category. Read the Instructions Manual for examples of generic use descriptions.
	N/A

☐ Mark (X) this box if you attach a continuation sheet.

3. Hazard Information -- Include in the notice a copy of reasonable facsimile of any hazard warning statement, label, material safety data sheet, or other information which will be provided to any person who is reasonably likely to be exposed to this substance regarding protective equipment or practices for the safe handling, transport, use, or disposal of the new substance. List in part III hazard information you include.	Binding Option Mark (x)
<input checked="" type="checkbox"/> Mark (X) this box if you attach hazard information. (MSDS)	

Part II-- HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE**Section A -- INDUSTRIAL SITES CONTROLLED BY THE SUBMITTER**

Mark (X) the "Confidential" box next to any item you claim as confidential

Complete section A for each type of manufacture, processing, or use operation involving the new chemical substance at industrial sites you control. Importers do not have to complete this section for operations outside the U.S.; however, you may still have reporting requirements if there are further industrial processing or use operations after import. You must describe these operations. See instructions manual

1. Operation description

a. Identity -- Enter the identity of the site at which the operation will occur.

Confidential

Name

Site address (number and street)

N/A

City, County, State, ZIP code

If the same operation will occur at more than one site, enter the number of sites. Identify the additional sites on a continuation sheet, and if any of the sites have significantly different production rates or operations, include all the information requested in this section for those sites as attachments.


☐ Mark (X) this box if you attach a continuation sheet.
b. Type --
Mark (X)

Manufacturing



Processing



Use

c. Amount and Duration -- Complete 1 or 2 as appropriate

1. Batch

Maximum kg/batch (100% new chemical substance)

Hours/batch

Batches/year

2. Continuous

Maximum kg/batch (100% new chemical substance)

Hours/batch

Batches/year

d. Process description ☐ Mark (X) to indicate your willingness to have your process description binding.

- (1) Diagram the major unit operation steps and chemical conversions. Include interim storage and transport containers (specify- e.g. 5 gallon pails, 55 gallon drum, rail car, tank truck, etc.).
- (2) Provide the identity, the approximate weight (by kg/day or kg/batch on a 100% new chemical substance basis), and entry point of all starting materials and feedstocks (including reactants, solvents, catalysts, etc.), and of all products, recycle streams, and wastes. Include cleaning chemicals (note frequency if not used daily or per batch.).
- (3) Identify by number the points of release, including small or intermittent releases, to the environment of the new chemical substance.

Not applicable

☐ Mark (X) this box if you attach a continuation sheet.

Part II-- HUMAN EXPOSURE AND ENVIRONMENTAL RELEASE -- Continued**Section A -- INDUSTRIAL SITES CONTROLLED BY THE SUBMITTER -- Continued**

- 2. Occupational Exposure --** You must make separate confidentiality claims for the description of worker activity, physical form of the new chemical substance, number of works exposed, and duration of activity. Mark (X) the "Confidential" box next to any item you claim as confidential.
- (1) -- Describe the activities (i.e. bag dumping, tote filling, unloading drums, sampling, cleaning, etc.) in which workers may be exposed to the substance.
- (2) -- Mark (X) this column if entry in column (1) is confidential business information (CBI).
- (3) -- Describe any protective equipment and engineering controls used to protect workers.
- (4) and (6) -- Indicate your willingness to have the information provided in column (3) or (5) binding.
- (5) -- Indicate the physical form(s) of the new chemical substance (e.g., solid; crystal, granule, powder, or dust) and % new chemical substance (if part of a mixture) at the time of exposure.
- (7) -- Mark (X) this column if entry in column (5) is confidential business information (CBI).
- (8) -- Estimate the maximum number of workers involved in each activity for all sites combined.
- (9) -- Mark (X) this column if entry in column (8) is confidential business information (CBI).
- (10) and (11) -- Estimate the maximum duration of the activity for any worker in hours per day and days per year.
- (12) -- Mark (X) this column if entries in columns (10) and (11) are confidential business information (CBI).

Worker activity (i.e., bag dumping, filling drums) (1)	CBI (2)	Protective Equipment/ Engineering Controls (3)	Binding Option Mark (x) (4)	Physical forms(s) and % new substance (5)	Binding Option Mark (x) (6)	CBI (7)	# of Workers Exposed (8)	CBI (9)	Maximum Hrs/day (10)	duration Days/yr (11)	CBI (12)
Not applicable		Not applicable									

☐ Mark (X) this box if you attach a continuation sheet.

- 3. Environmental Release and Disposal --** You must make separate confidentiality claims for the release number and the amount of the new chemical substance released and other release and disposal information. Mark (X) the "Confidential" box next to each item you claim as confidential.
- (1) -- Enter the number of each release point identified in the process description, part II, section A, subsection 1d(3).
- (2) -- Estimate the amount of the new substance released (a) directly to the environment or (b) into control technology (in kg/day or kg/batch).
- (3) -- Mark (X) this column if entries in columns (1) and (2) are confidential business information (CBI).
- (4) -- Identify the media (stack air, fugitive air (optional-see Instruction Manual), surface water, on-site or off-site land or incineration, POTW, or other (specify)) to which the new substance will be released from that release point.
- (5) -- a. Describe control technology, if any, and control efficiency that will be used to limit the release of the new substance to the environment. For releases disposed of on land, characterize the disposal method and state whether it is approved for disposal of RCRA hazardous waste. On a continuation sheet, for each site describe any additional disposal methods that will be used and whether the waste is subject to secondary or tertiary on-site treatment. b. Estimate the amount released to the environment after control technology (in kg/day).
- (6) -- Mark (X) this column if entries in columns (4) and (5) are confidential business information (CBI).
- (7) -- Identify the destination(s) of releases to water. Please supply NPDES (National Pollutant Discharge Elimination System) numbers for direct discharges or NPDES numbers of the POTW (Publicly Owned Treatment Works). Mark (X) if the POTW name or NPDES # is confidential business information (CBI).

Release Number (1)	Amount of new substance released		CBI (3)	Media of release e.g. stack air (4)	Control technology and efficiency (you may wish to optionally attach efficiency data)		Binding Mark (X)	(5b)	CBI (6)
	(2a)	(2b)			(5a)				
					Not applicable				
(7) Mark (X) the destination(s) of releases to water.		<input type="checkbox"/> POTW provide name(s) below: _____		CBI	<input type="checkbox"/> Navigable waterway	<input type="checkbox"/> Other - Specify	provide NPDES #		CBI

☐ Mark (X) this box if you attach a continuation sheet.

OPTIONAL POLLUTION PREVENTION INFORMATION

To claim information in this section as confidential circle or bracket the specific information that you claim as confidential.

In this section you may provide information not reported elsewhere in this form regarding your efforts to reduce or minimize potential risks associated with activities surrounding manufacturing, processing, use and disposal of the PMN substance. Please include new information pertinent to pollution prevention, including source reduction, recycling activities and safer processes or products available due to the new chemical substance. Source reduction includes the reduction in the amount or toxicity of chemical wastes by technological modification, process and procedure modification, product reformulation, raw materials substitution, and/or inventory control. Recycling refers to the reclamation of useful chemical components from wastes that would otherwise be treated or released as air emissions or water discharges, or land disposal. Descriptions of pollution prevention, source reduction and recycling should emphasize potential risk reduction subsequent to compliance with existing regulatory requirements and can be either quantitative or qualitative. The EPA is interested in the information to assess overall net reductions in toxicity or environmental releases and exposures, not the shifting of risks to other environmental media or non-environmental areas (e.g., occupational or consumer exposure). In addition, information on the relative cost or performance characteristics of the PMN substance to potential alternatives may be provided.

All information provided in this section will be taken into consideration during the review of this substance. See Instructions Manual and Pollution Prevention Guidance manual for guidance and examples.

Describe the expected net benefits, such as (1) an overall reduction in risk to human health or the environment; (2) a reduction in the volume manufactured; (3) a reduction in the generation of waste materials through recycling, source reduction or other means; (4) a reduction in potential toxicity or human exposure and/or environmental release; (5) an increase in product performance, a decrease in the cost of production and/or improved operation efficiency of the new chemical substance in comparison to existing chemical substances used in similar application; or (6) the extent to which the new chemical substance may be a substitute for an existing substance that poses a greater overall risk to human health or the environment.

[N/A]

☐ Mark (X) this box if you attach a continuation sheet.

Part III -- LIST OF ATTACHMENTS

Attach continuation sheets for sections of the form and test data and other data (including physical/chemical properties and structure/activity information), and optional information after this page. Clearly identify the attachment and the section of the form to which it relates, if appropriate. Number consecutively the pages of the attachments. In the column below, enter the inclusive page numbers of each attachment.

Mark (X) the "Confidential" box next to any attachment name you claim as confidential. Read the Instructions Manual for guidance on how to claim any information in an attachment as confidential. You must include with the sanitized copy of the notice form a sanitized version of any attachment in which you claim information as confidential.

Attachment name	Attachment page number(s)	Confidential
Continuation Sheet – Section 1 (e) Class 2 Substance	yes	X
Continuation Sheet - Part II Section B(1)	yes	X
Material Safety Data Sheet (MSDS)	Yes	X
Certificate of Analysis	Yes	X
Determination of General Physico-chemical Properties (Melting & boiling point, density, surface tension, water solubility, partition coefficient, particle-size)	Yes	X
Determination of Hazardous Physico-chemical Properties (Vapour pressure, flammability, explosive properties, self-ignition, oxidizing properties)	Yes	X
Determination of Spectra	Yes	X
Acute Oral Toxicity in Rats	Yes	X
Acute Dermal Irritation in Rabbits	Yes	X
Acute Eye Irritation in Rabbits	Yes	X
Local Lymph Node Assay in Mice (LLNA)	Yes	X
Bacterial Reverse Mutation Test	Yes	X
In vitro Mammalian Chromosome Aberration Test	Yes	X
Development and Validation of analytical method for the analysis SH-1 in vehicle	Yes	X
28-Day Repeated Dose Oral Tox Study Followed by 14 day recovery - Rat	Yes	X
Subacute 28-Day Oral Tox Study-Rat (METI Summary)	Yes	X
Acute Toxicity to Rainbow Trout	Yes	X
Acute Toxicity to Daphnia Magna	Yes	X
Toxicity to Activated Sludge in a Respiration Inhibition Test	Yes	X
Ready Biodegradability in a CO2 Evolution Test (Sturm)	Yes	X
Soil Adsorption	Yes	X
Algal Growth Inhibition Test	Yes	X

☐ Mark (X) this box if you attach a continuation sheet. Enter the attachment name and number.

PHYSICAL AND CHEMICAL PROPERTIES WORKSHEET

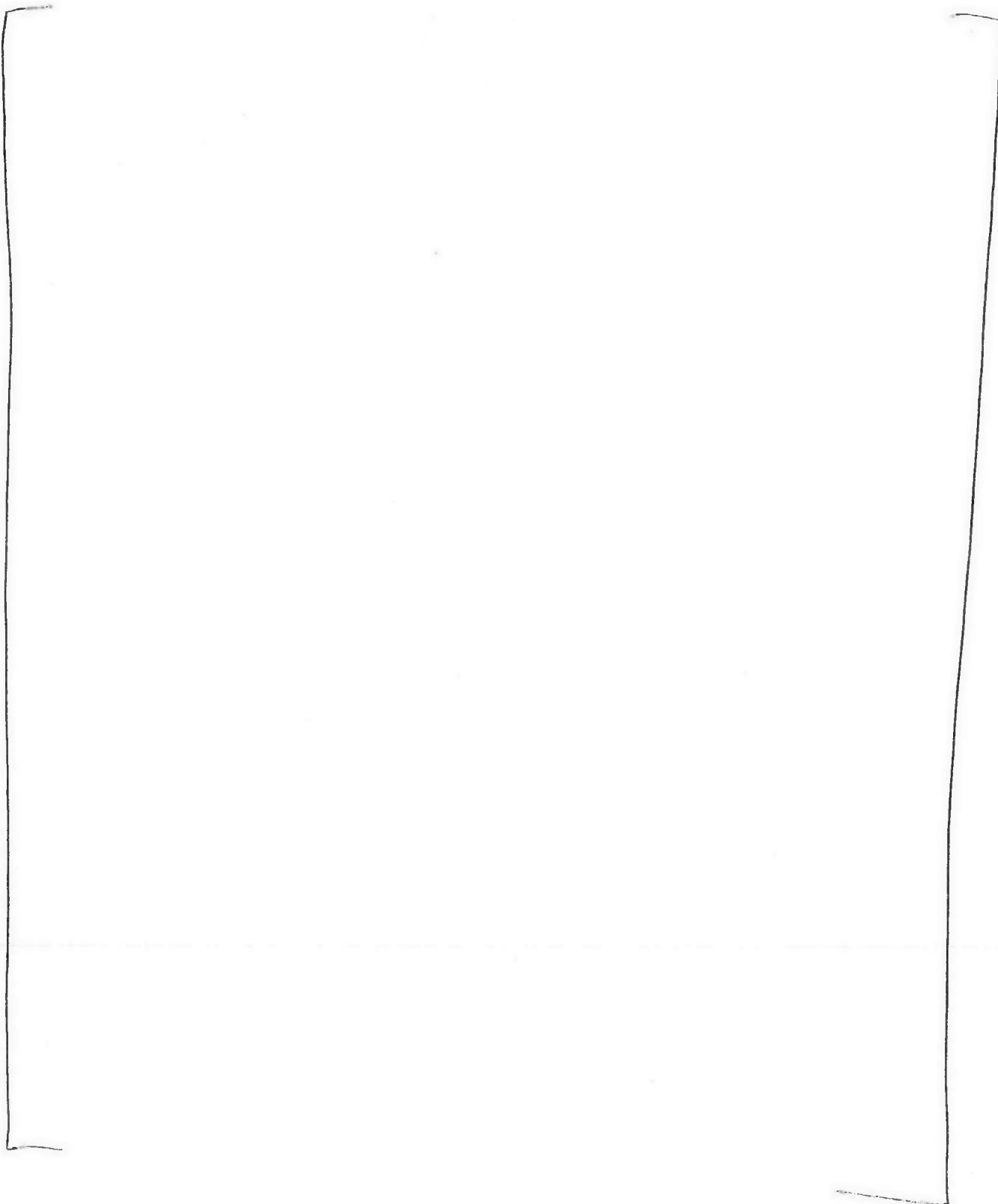
To assist EPA's review of physical and chemical properties data, please complete the following worksheet for data you provide and include it in the notice. Identify the property measured, the page of the notice on which the property appears, the value of the property, the units in which the property is measured (as necessary), and whether or not the property is claimed as confidential. The physical state of the neat substance should be provided. These measured properties should be for the neat (100% pure) chemical substance. Properties that are measured for mixtures or formulations should be so noted (% PMN substance in ____). You are not required to submit this worksheet; however, EPA strongly recommends that you do so, as it will simplify review and ensure that confidential information is properly protected. You should submit this worksheet as a supplement to your submission of test data. This worksheet is not a substitute for submission of test data.

Property (a)	Mark (X) if provided	Page numbe r (b)	Value (c)	Measured or Estimate (M or E)	Confidential Mark (X) (d)
Physical state of neat substance	X	[]	[]	M	X
Vapor pressure @ Temperature [] °C	X	[]	[]	M	X
Density/relative density @ [] °C	X	[]	[]	M	X
Solubility @ Temperature _____ °C Solvent _____					
Solubility in water @ Temperature [] °C	X	[]	[]	M	X
Melting temperature	X	[]	[]	M	X
Boiling / sublimation temperature @ _____ torr pressure	X	[]	[]	M	X
Spectra (GC, IR)	X	[]	[]	M	X
Dissociation constant					
Particle size distribution	X	[]	[]	M	X
Octanol / water partition coefficient	X	[]	[]	M	X
Henry's Law constant					
Volatilization from water					
Volatilization from soil					
pH @ concentration _____					
Flammability Flash Point	X	[]	[]	M	X
Explosibility	X	[]	[]	M	X
Adsorption / coefficient				M	
Other – Specify (Micro Analysis)					
Total base number					
Water (Karl Fischer)					

14

Continuation Sheet – Section 1 (e) Class 2 Substance
(1) List the immediate precursor substances, with their respective CASRN. (2) Describe the nature of the reaction or process. (3) Indicate the range of composition and the typical composition (where appropriate). (4) Provide a correct representative or partial chemical structure diagram as complete as can be known, if one can reasonably be ascertained.

Continuation Sheet – Section 1 (e) Class 2 Substance



Part II Section B(1) Continuation Sheet

Samsung Cheil Industries Inc.

ADDRESS : 62, Pyeongnyeo-dong, Yeosu-shi, Jeollanam-do, KOREA

Tel: 82-61-689-1531 Fax: 82-61-689-1535

Material Safety Data Sheet

Product Name: []

SECTION I - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name: []

Manufacturer: Samsung Cheil Industries Inc

Address: 62, Pyeongnyeo-dong, Yeosu-shi, Jeollanam-do, KOREA

Email: starex@samsung.com

Information Telephone Number: 82-61-689-1531 Fax: 82-61-689-1535

MSDS Prepared By: Quality Control Department/ Samsung Cheil Industries Inc

Synonyms: []

Product Use: Flame Retardant

Chemical Name: []

Chemical Family: Halogenated aromatic

Additional Information: No information available

SECTION II - COMPOSITION/INFORMATION ON INGREDIENTS

INGREDIENT NAME	CAS NO.	%	EXPOSURE LIMITS
[]	[]	[]	Y (Hazardous) 15 mg/m3 (PNOR) (OSHA PEL TWA) Not established (OSHA PEL STEL) Not established (OSHA PEL CEIL) 10 mg/m3 (PNOC) (ACGIH TLV TWA) Not established (ACGIH TLV STEL) Not established (ACGIH TLV CEIL)

*Indented chemicals are components of previous ingredient.

Additional Information

5 mg/m3 Respirable Dust Level (OSHA)

3 mg/m3 Respirable Dust Level (ACGIH)

PNOR = Particulates Not Otherwise Regulated

PNOC = Particulates Not Otherwise Classified

SECTION III - HAZARDS IDENTIFICATION

Emergency Overview: Off white powder or granules

No odor

The hazards of this material have not been fully investigated.

Not expected to be acutely toxic.

Relevant Routes of Exposure: Ingestion, inhalation and skin absorption

Signs and Symptoms of Overexposure: No known signs and symptoms of exposure.

Medical Conditions Generally Aggravated By Exposure: None known

Potential Health Effects: See Section XI for additional information.

Eyes: Not expected to be a hazard in normal industrial use.

As with any dust, mechanical irritation is possible to the eye.

SECTION III - HAZARDS IDENTIFICATION

Skin: Not expected to be acutely toxic.

As with any dust, mechanical irritation is possible to the skin. See Chronic Health Effects.

Ingestion: Not expected to be acutely toxic.

Inhalation: Not expected to be acutely toxic.

As with any dust, mechanical irritation is possible to mucous membranes and the respiratory tract. See Chronic Health Effects.

Chronic Health Effects: From data comparisons to similar compounds, the Environmental Protection Agency has concluded that this substance may cause cancer as a result of significant chronic dermal and inhalation exposures to workers.

Carcinogenicity:

NTP: No

IARC: No

ACGIH: No

OTHER: No

OSHA: No

Additional Information: No information available

SECTION IV - FIRST AID MEASURES

Eyes: Flush with large volumes of water for at least 15 minutes. Get medical attention.

Skin: Wash with large volumes of soap and water for at least 15 minutes. If irritation develops, get medical attention.

Ingestion: If conscious, give person 1 to 2 glasses of water. Get medical attention immediately

Inhalation: Remove person to fresh air. Get medical attention.

Antidotes: No information available

Notes to Physicians and/or Protection for First-Aiders: No information available

Additional Information No information available

SECTION V - FIRE FIGHTING MEASURES

Flammable Limits in Air (% by Volume): Not available

Flash Point: Not applicable

Autoignition Temperature: Not available

Extinguishing Media: All conventional media are suitable.

Fire Fighting Instructions: Wear a selfcontained breathing apparatus and protective clothing to prevent skin and eye contact in fire situations.

Unusual Fire and Explosion

Hazards: Under fire conditions, toxic and irritating fumes may be emitted.

Flammability Classification: Non-flammable solid

Known or Anticipated Hazardous Products of : Hydrogen bromide and/or bromine

Combustion: Carbon monoxide and carbon dioxide

Additional Information: No information available

SECTION VI - ACCIDENTAL RELEASE MEASURES

Accidental Release Measures: Wearing appropriate personal protective equipment, carefully sweep up material and place in suitable labeled containers for disposal.

Personal Precautions: See Section VIII.

Environmental Precautions: Based on comparison to similar compounds, the Environmental Protection Agency has concluded that this compound may be toxic to aquatic organisms. Do not release this compound into U.S. waters.

Additional Information No information available

SECTION VII - HANDLING AND STORAGE

Handling: Use appropriate personal protection equipment.

Avoid eye, skin and clothing contact. Avoid breathing dust.

Avoid repeated and prolonged contact.

Avoid creating a dusting situation.

Storage: Store in a cool, dry, well-ventilated area away from incompatible materials. Keep container tightly closed.

Other Precautions: No information available

Additional Information No information available

SECTION VIII - EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Controls: No information available

Ventilation Requirements: Use local exhaust to minimize dusting.

Use mechanical ventilation for general area control.

Personal Protective Equipment:

Eye/Face Protection: Chemical safety glasses with side shields or chemical safety goggles

Skin Protection: Rubber gloves Clothing designed to minimize skin contact

Respiratory Protection: When using this compound and exposure through inhalation is likely,

wear a NIOSH/MSHA approved Category 21C air-purifying respirator equipped with a full face piece and high efficiency particulate filters or a Category 21C powered air-purifying respiratory equipped with a tight-fitting facepiece and high efficiency particulate filters. Consult the OSHA respiratory protection information located at 29CFR 1910.134 and the American National Standard Institute's Practices of Respiratory Protection Z88.2.

Other Protective

Clothing or Equipment: No information available

Exposure Guidelines: See Section II.

Work Hygienic Practices: Wash thoroughly after handling.
Wash contaminated clothing before reuse.

Additional Information No information available

SECTION IX - PHYSICAL & CHEMICAL PROPERTIES

Appearance: Off white powder or granules

Boiling Point: Not available

Bulk Density: Not available

Color: Off white **Physical State:** Solid

Reactivity in Water: Not water reactive

Saturated Vapor Concentration: Not available

Freezing Point: Not available

Heat Value: Not available

Melting Point: 170 degrees C minimum

Specific Gravity or Density (Water=1): Not available

Molecular/Chemical Formula: []

Molecular Weight: []

Octanol/Water Partition Coefficient: Not available

Odor: None

Odor Threshold: Not available

Particle Size: See Below

Percent Volatile: Not available

pH Value: Not available

pH Concentration: Not available

Decomposition Temperature: >300C

Evaporation Rate: Not available

Softening Point: Not available

Solubility in Water: Insoluble

Vapor Density: Not available

Vapor Pressure: Not available

Viscosity: Not available

Volatile Organic Compounds: Not available

Water/Oil Distribution Coefficient: Not available

Weight Per Gallon: Not available

Additional Information:

SECTION X - STABILITY AND REACTIVITY

Stability: Stable under normal conditions of handling and use.

Conditions to Avoid: None

Incompatibility With Other Materials: Strong oxidizers

Hazardous Decomposition Products: Thermal decomposition may produce the following:

Hydrogen bromide and/or bromine

Carbon monoxide and carbon dioxide

Hazardous Polymerization: Will not occur

Conditions to Avoid: None

Additional Information No information available

SECTION XI - TOXICOLOGICAL INFORMATION**VALUE ANIMAL ROUTES COMPONENTS :** None**Toxicological Information:** Unknown**SECTION XI - TOXICOLOGICAL INFORMATION****Additional Information** No information available**SECTION XII - ECOLOGICAL INFORMATION****Ecological Information:** Unknown**Additional Information** No information available**SECTION XIII - DISPOSAL CONSIDERATIONS****Disposal Considerations:** Dispose of waste at an approved chemical disposal facility in compliance with all current Local, State/Province, Federal/Canadian laws and regulations.**Additional Information** No information available**SECTION XIV - TRANSPORT INFORMATION****U.S. DOT****Proper Shipping Name:** Not regulated**ID Number:** N/A**Labels:** N/A**Packaging Exceptions:** N/A**Bulk Packaging:** N/A**Air Cargo Limit:** N/A**Other Stowage:** N/A**Hazard Class:** N/A**Packing Group:** N/A**Special Provisions:** N/A**Non-Bulk Packaging:** N/A**Passenger Air/Rail Limit:** N/A**Vessel Stowage:** N/A**Reportable Quantity:** N/A**AIR - ICAO OR IATA****Proper Shipping Name:** Not regulated**Hazard Class:** N/A**Subsidiary Risk:** N/A**Hazard Labels:** N/A**Air Passenger Limit Per Package:** N/A**Air Cargo Limit Per Package:** N/A**ID Number:** N/A**Packing Group:** N/A**Packing Instructions:** N/A**Packing Instruction - Cargo:** N/A**Special Provisions Code:** N/A**WATER - IMDG****Proper Shipping Name:** Not regulated**Hazard Class:** N/A **ID Number:** N/A**Subsidiary Risk:** N/A**Packing Group:** N/A**Medical First Aid Guide Code:** N/A**Additional Information** No information available

SECTION XV - REGULATORY INFORMATION

U.S. Federal Regulations:

The components of this product are either on the TSCA Inventory or exempt (i.e. impurities, a polymer complying with the exemption rule at 40 CFR 723.250) from the Inventory.

The manufacture and use of this material is regulated by a Significant New Use Rule (SNUR) under TSCA which can be located at []

State Regulations: None known

International Regulations:

This material (or each component) is listed on the following inventories:

EU - EINECS

Canadian WHMIS Hazard Class and Division = Not controlled

SARA Hazards:

Acute: No

Chronic: No

Reactive: No

Fire: No

Pressure: No

Additional Information : No information available

SECTION XVI - OTHER INFORMATION

NFPA Codes:

Health: 1

Flammability: 0

Reactivity: 0

Other: N

HMIS Codes: * indicates chronic health hazard.

Health: 1*

Flammability: 0

Reactivity: 0

Protection: X

Label Statements: Not available

Other Information: Abbreviations:

(L) = Loose bulk density in g/ml

LOEC = Lowest observed effect concentration

MATC = Maximum acceptable toxicant concentration

NA = Not available

N/A = Not applicable

NL = Not limited

NOAEL = No observable adverse effect level

NOEC = No observed effect concentration

NOEL = No observable effect level

NR = Not rated

(P) = Packed bulk density in g/ml

PNOC = Particulates Not Otherwise Classified

PNOR = Particulates Not Otherwise Regulated

REL = Recommended exposure limit

TS = Trade secret

Information is supplied upon the condition that the persons receiving same will make their own determination as to its safety and suitability for their purposes prior to use. In no event will Samsung Cheil Industries Inc be responsible for damages of any nature whatsoever resulting from the use of or reliance upon information.

Page: 7 of 7

End of document

Appendix 4 Certificate of Analysis

Certificate of Analysis

1. TEST MATERIAL IDENTIFICATION

- 1) PRODUCT NAME : []
2) TEST MATERIAL(LOT No.) : 20090105
3) QUANTITY : 100g
4) CHEMICAL NAME : []
5) COMPOSITIONS OF CHEMICAL

Chemical name	CAS No.	Contents

- 6) PURITY : >99%
7) APPEARANCE : White Powder
8) MOLECULAR WEIGHT : []
9) MOLECULAR FORMULA : []

2. SOLUBILITY :

- 1) INSOLUBLE : H₂O
2) SOLUBLE : Toluene(Partially)

3. STORAGE :

- 1) Storage temperature : Room Temperature
2) Expiry date : Jan. 08. 2011

We hereby certify that the data stated here above are true and correct

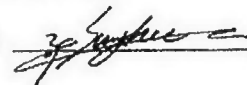
Company(Manufacturer) : CHEIL INDUSTRIAL INC.

Address : (437-711) 332-2, Gocheon-dong, Uiwang-si, Gyeonggi-do, Korea

Date : Jan. 09. 2009

Name of person in charge

Sunghee Ahn



**DETERMINATION OF GENERAL
PHYSICO-CHEMICAL PROPERTIES**

PROJECT NUMBER: 2737/0004

AUTHORS: R E Butler
J A Walker
D F White

STUDY SPONSOR:

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Shardlow Business Park
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QUALITY ASSURANCE REPORT

This study type is classed as short-term. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§ 09 February 2009	Protocol Compliance Audit
06 February 2009	Melting Temperature
09, 11 March 2009	Boiling Temperature
13 March 2009	Relative Density
05 May 2009	Water Solubility
02 March 2009	Partition Coefficient
03 March 2009	Particle Size Distribution
§ 25 June 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... G. Wren DATE: 07 JUL 2009
For the Quality Assurance Unit*

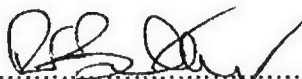
***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.



DATE: - 6 JUL 2009

R E Butler GRSC
STUDY DIRECTOR

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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DETERMINATION OF GENERAL PHYSICO-CHEMICAL PROPERTIES

SUMMARY

Melting/Freezing Temperature. The test material has been determined to melt in the range 412 to 499 K, by differential scanning calorimetry, using ASTM E537-86, Method A1 Melting/Freezing Temperature of Commission Regulation (EC) No 440/2008 of 30 May 2008.

Boiling Temperature. Greater than 673 ± 0.5 K at 101.51 kPa, by differential scanning calorimetry, using ASTM E537-86, Method A2 Boiling Temperature of Commission Regulation (EC) No 440/2008 of 30 May 2008.

Relative Density. 2.68 at $21.0 \pm 0.5^\circ\text{C}$, using a gas comparison pycnometer, Method A3 Relative Density of Commission Regulation (EC) No 440/2008 of 30 May 2008.

Surface Tension. The surface tension was not determined according to Method A5 Surface Tension of Commission Regulation (EC) No 440/2008 of 30 May 2008 as the water solubility was determined to be less than 1 mg/l. The method guideline states that substances with a water solubility of less than 1 mg/l need not be tested for surface tension.

Water Solubility. Less than 4.36×10^{-5} g/l of solution at $20.0 \pm 0.5^\circ\text{C}$, using the column elution method, Method A6 Water Solubility of Commission Regulation (EC) No 440/2008 of 30 May 2008.

Partition Coefficient. Greater than 3.16×10^6 , $\log_{10} P_{ow} > 6.5$, using the HPLC method, Method A8 Partition Coefficient of Commission Regulation (EC) No 440/2008 of 30 May 2008.

Particle Size Distribution. Particle size data have been acquired using a procedure designed to comply with the European Commission technical guidance document 'Particle Size Distribution, Fibre Length and Diameter Distribution' (June 1996), which satisfies the requirements of OECD Guideline 110. The results are as follows:

Measurement	Method	Result
Proportion of test material having an inhalable particle size less than 100 µm	Sieve	19.1 %
Proportion of test material having a thoracic particle size less than 10.0 µm	Cascade Impactor	0.926 %
Proportion of test material having a respirable particle size less than 5.5 µm	Cascade Impactor	0.263 %

DETERMINATION OF GENERAL PHYSICO-CHEMICAL PROPERTIES

1. INTRODUCTION

General physico-chemical properties of the test material have been determined.

Methods employed complied with those specified in Commission Regulation (EC) No 440/2008 of 30 May 2008, Part A: Methods for the determination of physico-chemical properties and 'Particle Size Distribution, Fibre Length and Diameter Distribution' (June 1996, European Commission technical guidance document), which satisfies the requirements of OECD Guideline 110.

Testing was conducted between 09 February 2009 and 02 June 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification :

Description : white powder

Batch number : 20090105

Date received : 23 January 2009

Storage conditions : room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

3. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

4. MELTING/FREEZING TEMPERATURE

4.1 Method

The determination was carried out by differential scanning calorimetry (DSC) using the procedure specified in ASTM E537-86, Method A1 Melting/Freezing Temperature of Commission Regulation (EC) No 440/2008 of 30 May 2008.

4.2 Procedure

4.2.1 Calibration

The temperature accuracy of the DSC was assessed using an indium reference standard (purity*99.999 %). The melting temperature was determined to be in the range 156.49 to 156.60°C and within the defined tolerance ($156.6 \pm 0.5^\circ\text{C}$). The DSC was therefore considered acceptable for use.

4.2.2 Sample

Aliquots (see following table) of test material were placed in pierced aluminium crucibles.

Table 4.1

Determination	Mass Taken (g)
1	0.0041
2	0.0058

4.2.3 Analysis

The DSC parameters were as follows:

Calorimeter : Mettler Toledo DSC822^e
ThermoHaake Cooler EK45/MT

Temperature program : initial: 25°C
rate: 5°C/min
final: 360°C

Atmosphere : air (static) Determination 1
nitrogen Determination 2

* Value quoted by supplier

4.3 Calculation

Measured temperatures were converted from °C to K using Equation 4.1.

Equation 4.1

$$T = t + 273.15$$

where:

T = temperature (K)

t = temperature (°C)

4.4 Results

Thermograms and thermographic data for Determinations 1 and 2 are shown in Figure 4.1 and Figure 4.2 and in the following tables respectively.

Figure 4.1 Thermogram – Determination 1

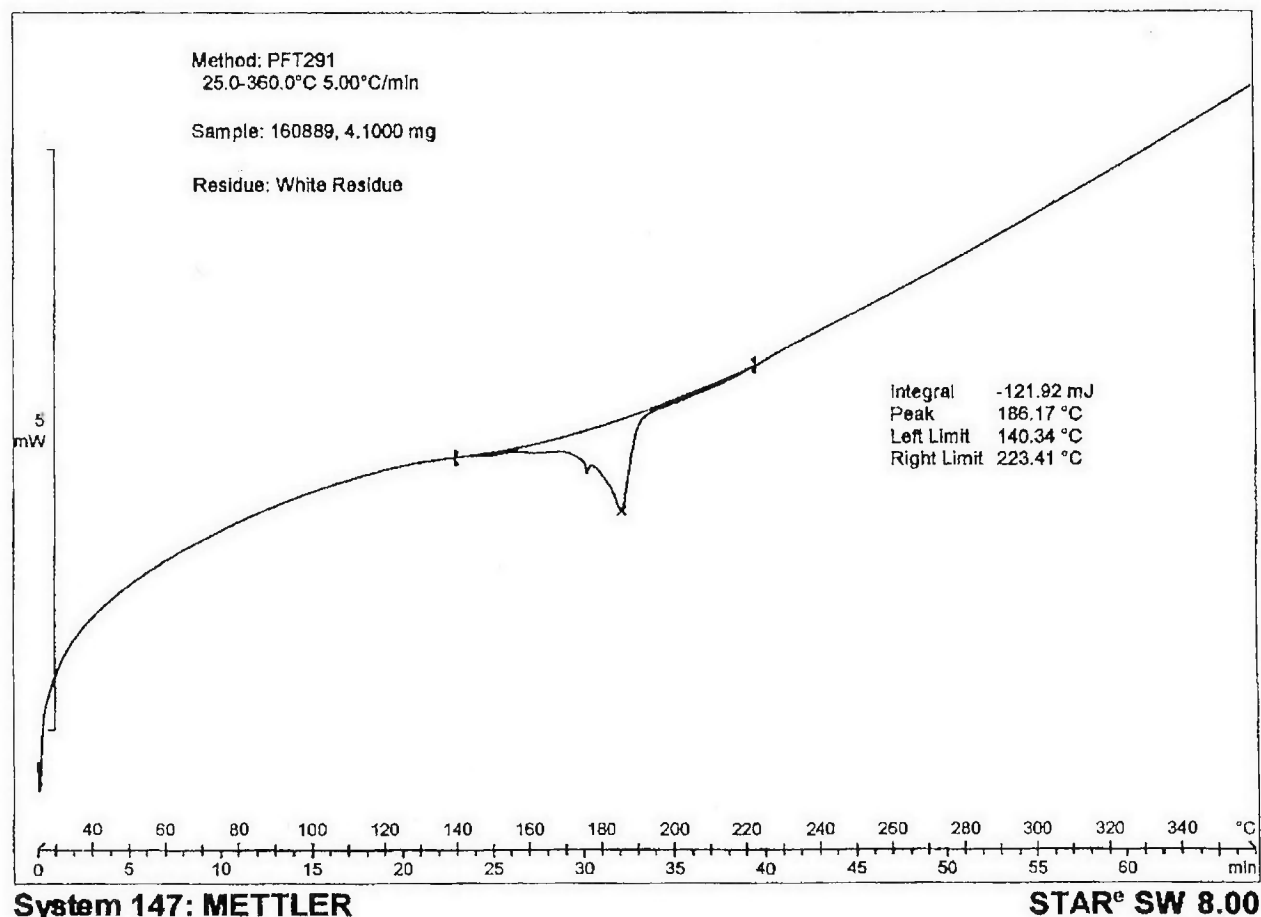
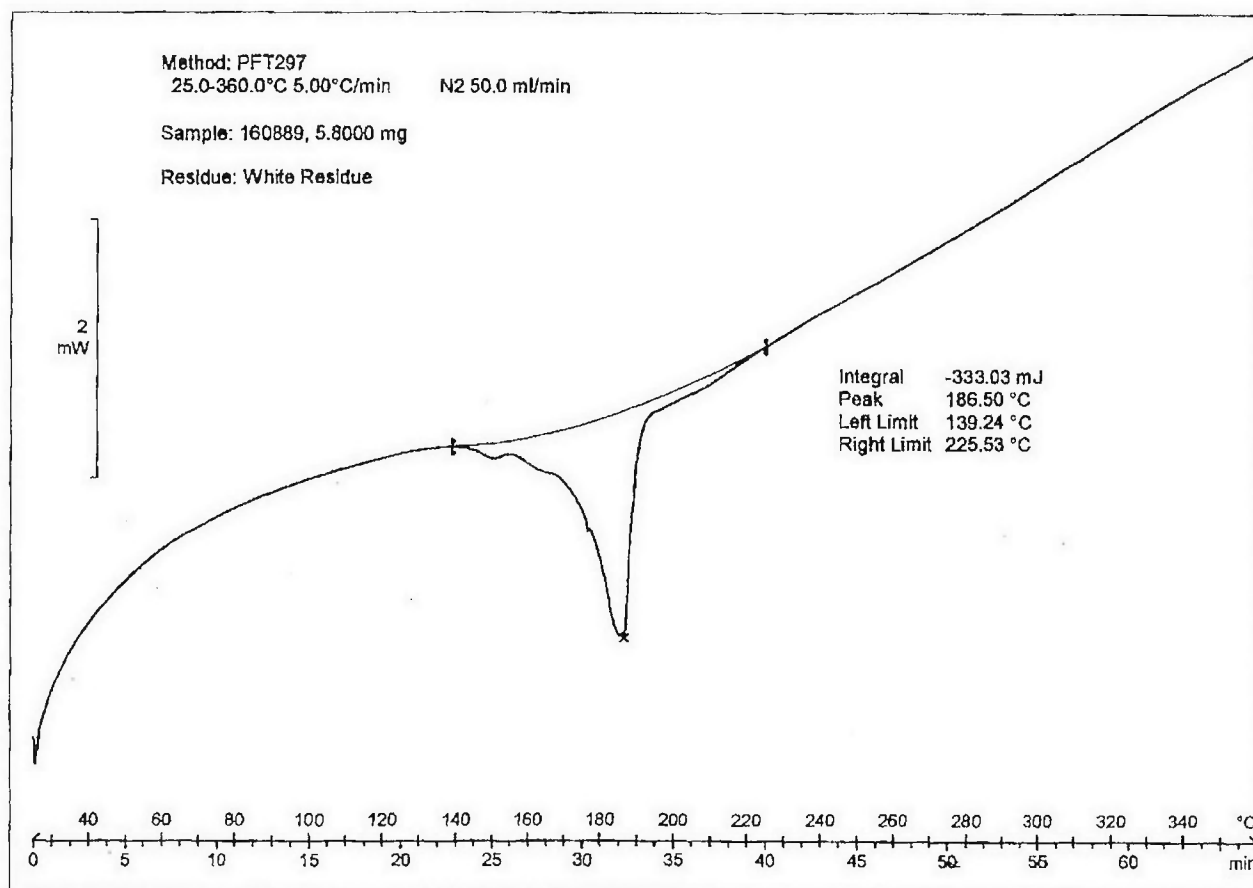


Table 4.2 Thermographic Data – Determination 1

Thermal Event	Interpretation	Temperature	
		°C	K
Endotherm	Onset of melting	140.34	413.49
	Peak melting	186.17	459.32
	End of melting	223.41	496.56

Figure 4.2 Thermogram – Determination 2



System 147: METTLER

Table 4.3 Thermographic Data – Determination 2

Thermal Event	Interpretation	Temperature	
		°C	K
Endotherm	Onset of melting	139.24	412.39
	Peak melting	186.50	459.65
	End of melting	225.53	498.68

Overall melting temperature: in the range 412 to 499 K.

4.5 Discussion

From compositional information supplied by the Sponsor, the test material was noted to consist of [] thus, it was considered that the complex melting results were typical for this substance.

The DSC results were confirmed using a metal block test, whereupon the test material visually started to melt at 181°C (454 K) and was fully molten at 224°C (497 K).

4.6 Conclusion

The test material has been determined to melt in the range 412 to 499 K.

5. BOILING TEMPERATURE

5.1 Method

The determination was carried out by differential scanning calorimetry (DSC) using the procedure specified in ASTM E537-86, Method A2 Boiling Temperature of Commission Regulation (EC) No 440/2008 of 30 May 2008.

5.2 Procedure

5.2.1 Calibration

The temperature accuracy of the differential scanning calorimeter was assessed using an indium reference standard (purity*99.999%). The melting temperature was determined to be 156.50°C and within the defined tolerance ($156.6 \pm 0.5^\circ\text{C}$). The instrument was therefore considered acceptable for use.

5.2.2 Sample

Aliquots (see following table) of test material were placed in perforated aluminium crucibles.

Table 5.1

Determination	Mass Taken (g)
1	0.0057
2	0.0052

* Value quoted by supplier

5.2.3 Analysis

The differential scanning calorimeter parameters were as follows:

Calorimeter : Mettler Toledo DSC822^e
ThermoHaake Cooler EK45/MT
Temperature program : initial: 25°C
rate: 20°C/min
final: 400°C
Atmosphere : air (static)

5.3 Calculation

Measured temperatures were converted from °C to K using Equation 5.1.

Equation 5.1

$$T = t + 273.15$$

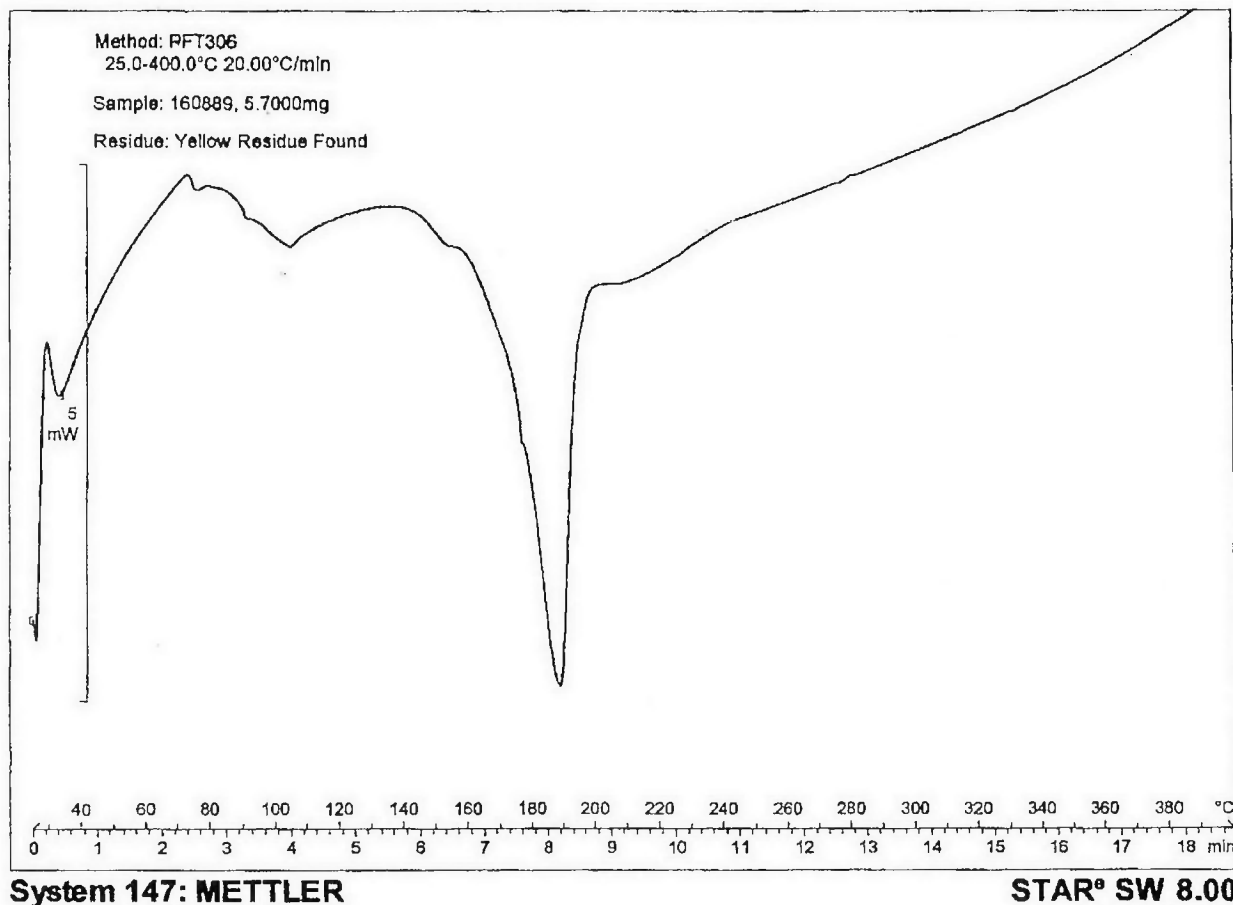
where:

T = temperature (K)
t = temperature (°C)

5.4 Results

Thermograms for determinations 1 and 2 are shown in Figure 5.1 and Figure 5.2 respectively.

Figure 5.1 Thermogram – Determination 1

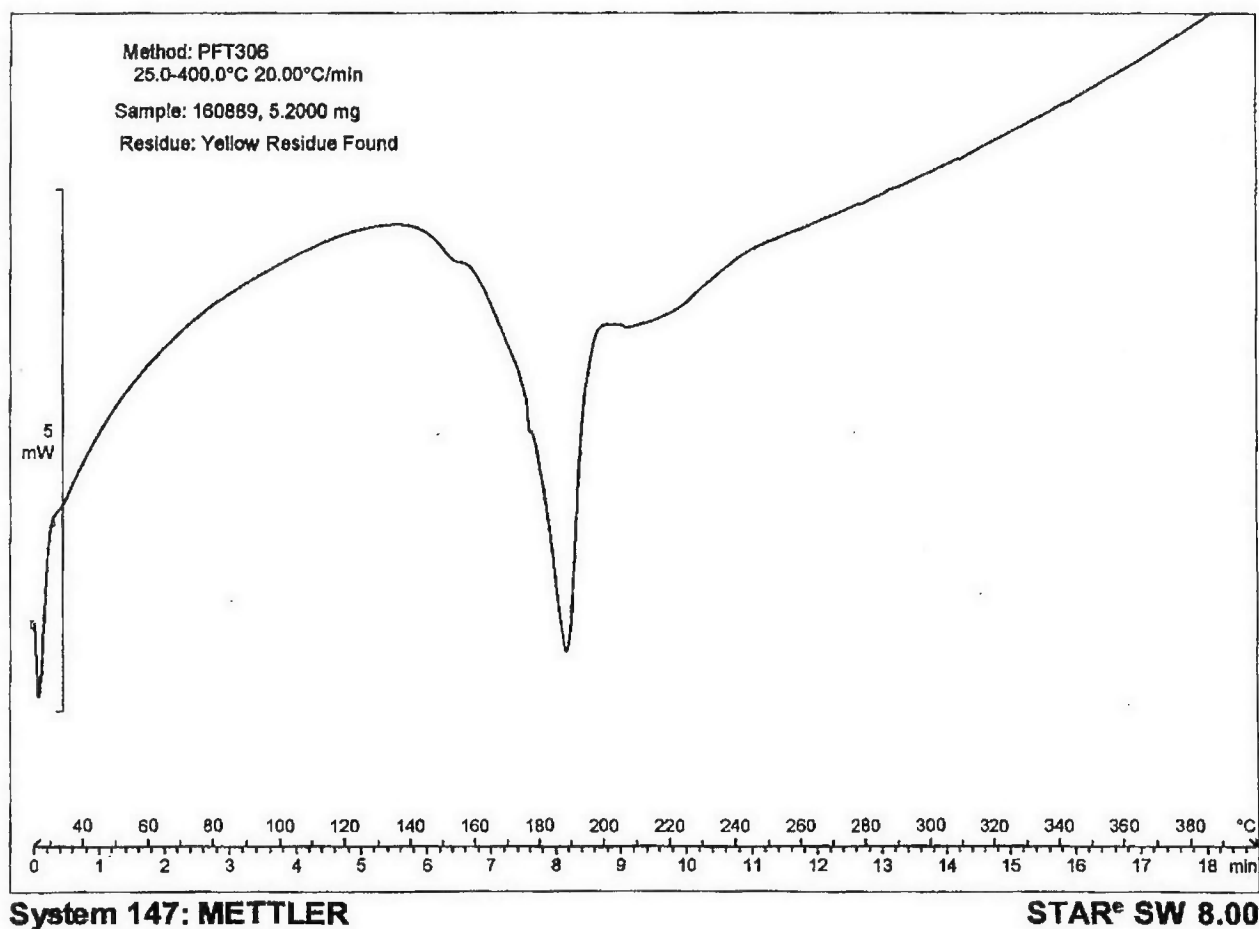


Atmospheric pressure: 101.51 kPa

Table 5.2 Thermographic Data – Determination 1

Thermal Event	Interpretation	Temperature	
		°C	K
-	No definitive signs of boiling	-	-

Figure 5.2 Thermogram – Determination 2



Atmospheric pressure: 101.51 kPa

Table 5.3 Thermographic Data – Determination 2

Thermal Event	Interpretation	Temperature	
		°C	K
-	No definitive signs of boiling	-	-

Overall boiling temperature: greater than 673 ± 0.5 K

5.5 Discussion

The endotherm at approximately 180°C was considered to be due to melting (see Section 4); no additional thermal events were detected.

A determination at reduced pressure was not performed; experience has shown that no further useful information is obtained for solid test materials which decompose prior to boiling at atmospheric pressure.

The residues noted in the melting temperature test [up to and including 360°C (633 K)] (see Section 4), were white, whereas the residues noted above [up to and including 400°C (673 K)] were yellow; thus, it was considered that some decomposition occurred within the range 633 to 673 \pm 0.5 K.

No value for the boiling temperature could be determined experimentally. However, using an adaptation of the Stein and Brown method, MPBPWIN version 1.43, September 2008, ©2000 US Environmental Protection Agency, the boiling temperature (based on the lowest molecular weight component) was calculated to be 721 K.

5.6 Conclusion

The test material has been determined to have a boiling temperature greater than 673 \pm 0.5 K at 101.51 kPa.

6. RELATIVE DENSITY

6.1 Method

The determination was carried out using a gas comparison pycnometer, Method A3 Relative Density of Commission Regulation (EC) No 440/2008 of 30 May 2008.

6.2 Procedure

Testing was carried out using a Quantachrome MVP-2 gas comparison pycnometer.

6.2.1 Calibration

A stainless steel test ball of known volume was used to calibrate the instrument prior to measurement of the test sample.

6.2.2 Sample

Aliquots (see following table) of test material were weighed into the sample cell of known volume (V_C), and placed into the pycnometer.

Table 6.1

Determination	Mass Taken (g)
A	85.6490
B	92.6073
C	97.4039
D	101.9056

Pressure readings (P_1 and P_2) were taken after pressurising the reference cell of known volume (V_R) and then switching to the sample cell (V_C).

6.3 Calculations

The relative density was calculated using Equation 6.1 to Equation 6.3.

Equation 6.1

$$V = V_C - V_R [(P_1 / P_2) - 1]$$

Equation 6.2

$$\rho = \frac{1000 m}{V}$$

Equation 6.3

$$\text{Relative density} = \frac{\rho}{\rho_{\text{H}_2\text{O}, 4^\circ\text{C}}}$$

where:

V	=	volume of test sample (cm^3)
V_C	=	volume of sample cell (149.225 cm^3)
V_R	=	volume of reference cell (90.953 cm^3)
P_1	=	pressure reading after pressurising reference cell (V_R)
P_2	=	pressure reading after switching to the sample cell (V_C)
ρ	=	density of test material (kg/m^3)
m	=	mass of test material taken (g)
$\rho_{\text{H}_2\text{O}, 4^\circ\text{C}}$	=	density of water at 4°C (999.97 kg/m^3)

6.4 Results

6.4.1 Calibration

The pressure readings (P_1 and P_2) and the calculated volume for the calibration ball are shown in the following table:

Table 6.2

Determination	P_1	P_2	Volume (cm^3)	Certified Volume (cm^3)	Tolerance (cm^3)
A	17.152	8.494	56.516	56.6	± 0.5
B	17.161	8.498	56.506	56.6	± 0.5
C	17.175	8.504	56.486	56.6	± 0.5
D	17.413	8.623	56.511	56.6	± 0.5

6.4.2 Sample

The pressure readings, calculated volumes and density values obtained for the test material are shown in the following table:

Table 6.3

Determination	P_1	P_2	Volume (cm^3)	Density (kg/m^3)
A	16.982	7.417	31.932	2.68×10^3
B	17.178	7.598	34.546	2.68×10^3
C	17.162	7.660	36.401	2.68×10^3
D	17.167	7.725	38.056	2.68×10^3

Temperature: $21.0 \pm 0.5^\circ\text{C}$

Mean density: $2.68 \times 10^3 \text{ kg/m}^3$

Relative density: 2.68

6.5 Conclusion

The relative density of the test material has been determined to be 2.68 at $21.0 \pm 0.5^\circ\text{C}$.

7. SURFACE TENSION

7.1 Summary

The surface tension was not determined according to Method A5 Surface Tension of Commission Regulation (EC) No 440/2008 of 30 May 2008 as the water solubility was determined to be less than 1 mg/l. The method guideline states that substances with a water solubility of less than 1 mg/l need not be tested for surface tension.

8. WATER SOLUBILITY

8.1 Method

The determination was carried out using the column elution method with a recirculating pump, Method A6 Water Solubility of Commission Regulation (EC) No 440/2008 of 30 May 2008.

8.2 Procedure

8.2.1 Preliminary test

An aliquot (0.1010 g) of test material was diluted to 1000 ml with glass double-distilled water. After shaking at 30°C for 3 hours and standing at 20°C for 18¼ hours, the solution was centrifuged at 13500 rpm for 15 minutes and analysed.

8.2.2 Definitive test

An aliquot (0.1050 g for systems A and B and 0.1019 g for systems C and D) of test material was dissolved in tetrahydrofuran (100 ml). Glass beads (1.0 g) were added and the solvent removed using a rotary evaporator.

The elution apparatus was set up in quadruplicate (systems A, B, C and D). Each system consisted of a glass micro-column fitted with a plug of glass wool, connected to a recirculating pump and a reservoir capable of holding approximately 2000 ml of water. The circulating water was maintained at $20.0 \pm 0.5^{\circ}\text{C}$ by means of a water bath fitted with a cooling coil.

The systems were flushed for at least 2 hours with distilled water which was then discarded. The reservoirs were re-filled with fresh glass double-distilled water and approximately half the coated glass beads loaded into each micro-column. After allowing the coated beads to soak for at least 2 hours, the water in the columns was run to waste. The recirculating pumps were switched on and approximately 25 ml of eluate collected to determine the flow rate for each system. These eluates were discarded and the water circulated through the columns.

Aliquots (100 ml) of sample solution from each reservoir were taken at intervals of at least ten bed volumes of eluate.

The pH of each solution was measured.

The flow of the circulated glass double-distilled water through each system is shown in the following table:

Table 8.1

Column	Flow Rate (ml/hour)
A	13.0
B	25.0
C	~3
D	~5

8.2.3 Analysis of sample solution

The concentration of test material in the sample solutions was determined by gas chromatography (GC).

Standard blank (Internal Standard Solution)

1,2,4-trichlorobenzene, at a nominal concentration of 10.0 mg/l in tetrahydrofuran.

Standards

Duplicate standard solutions were prepared in internal standard solution at a nominal concentration of 10 mg/l.

Samples

Each aliquot (100 ml) was frozen and freeze-dried. The residue was re-dissolved in 2 x 5 ml of tetrahydrofuran, transferred into a glass tube, evaporated to dryness using a nitrogen stream and re-dissolved in 1 ml of internal standard solution.

Sample blank

An aliquot of glass double-distilled water (100 ml) was frozen and freeze-dried. The residue was re-dissolved in 2 x 5 ml of tetrahydrofuran, transferred into a glass tube, evaporated to dryness using a nitrogen stream and re-dissolved in 1 ml of internal standard solution.

Analysis

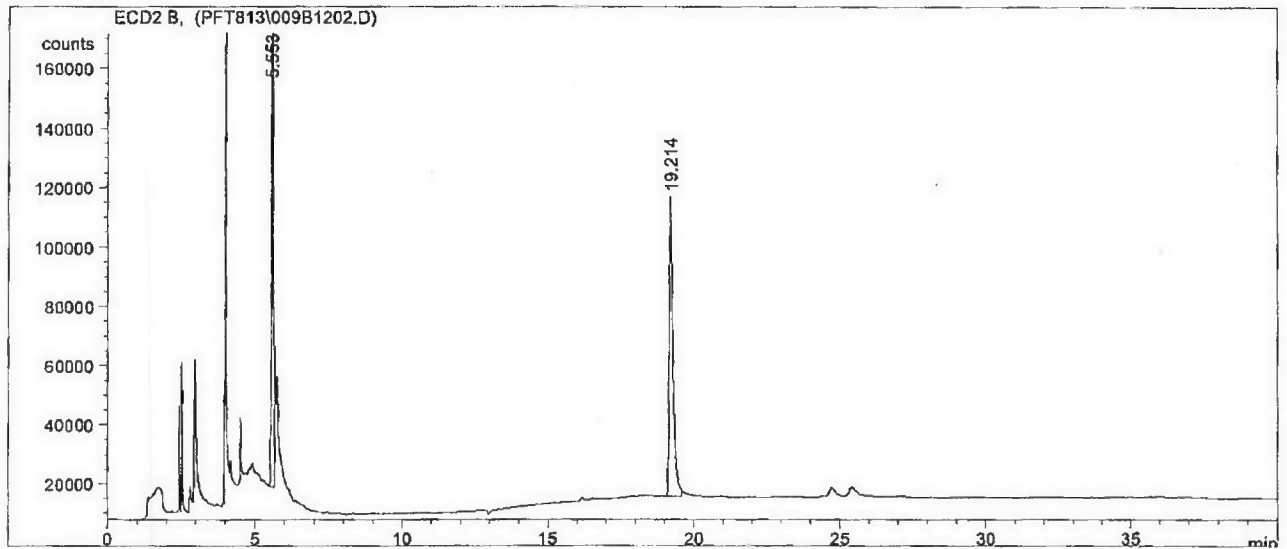
The standard and sample solutions were analysed by GC using the following typical conditions:

GC System	:	Agilent Technologies 5890, incorporating autosampler and workstation
Column	:	DB-5 (30 m x 0.25 mm id x 0.25 µm film)
Oven temperature program	:	initial 50°C for 2 mins* rate 40°C/min until 200°C rate 10°C/min until 260°C rate 20°C/min final 300°C
Injection Mode	:	splitless
Carrier gas pressure	:	~10 psi
Injection temperature	:	300°C
Electron capture detector temperature	:	310°C
Injection volume	:	1 µl
Retention time	:	~ 19 mins

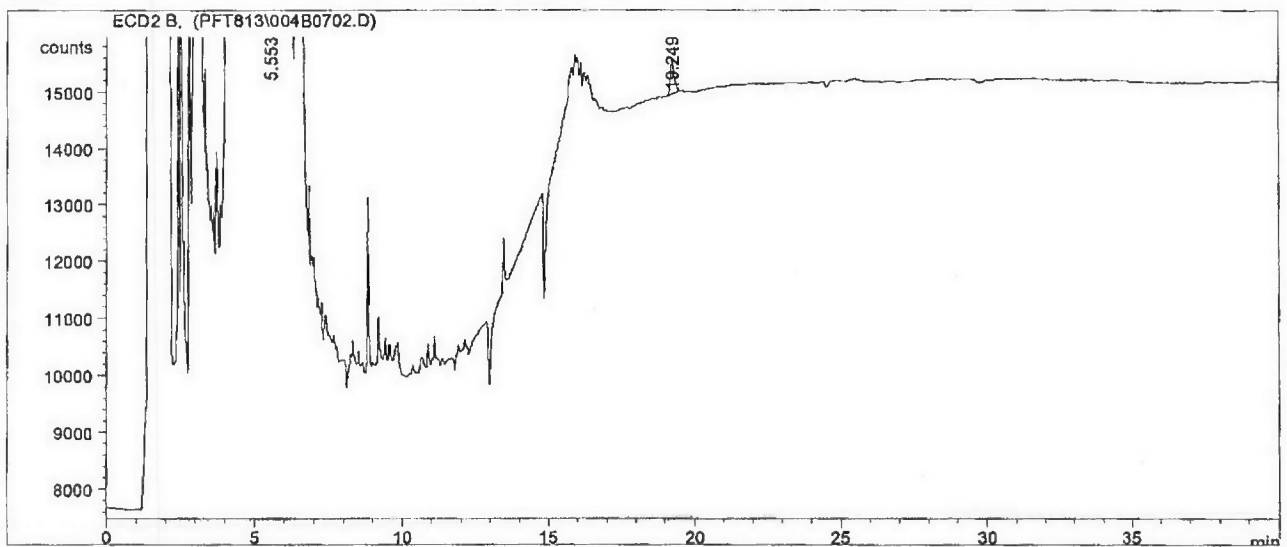
* For one analysis, the initial temperature was held for 1 minute. This was considered not to have a significant impact on the analysis.

Typical Chromatography

Standard 10.1 mg/l

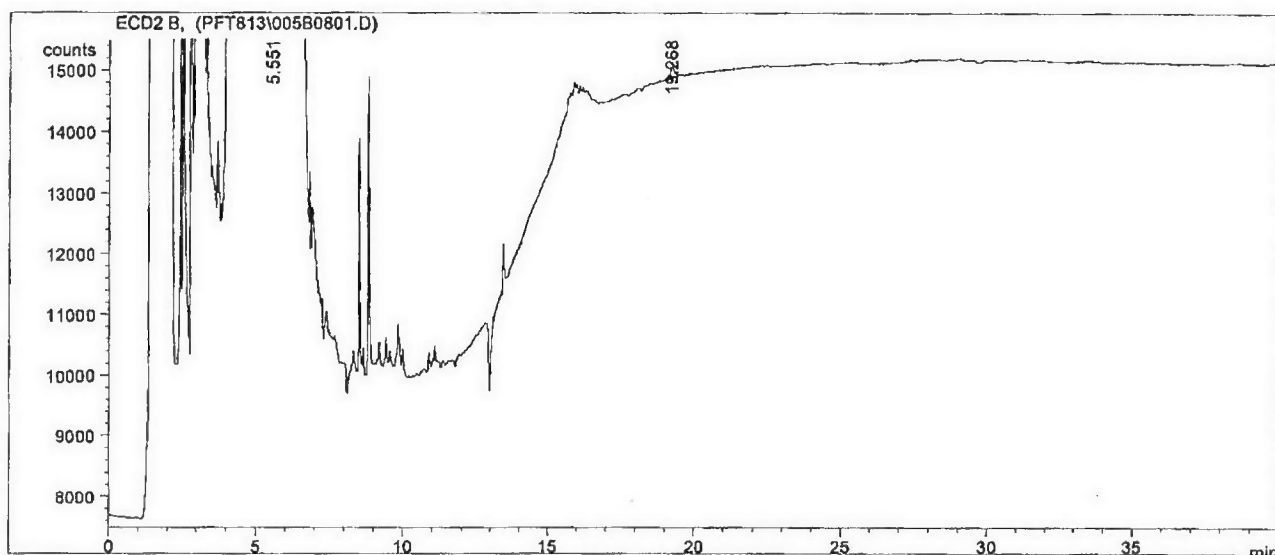


Determination A, Sample 1

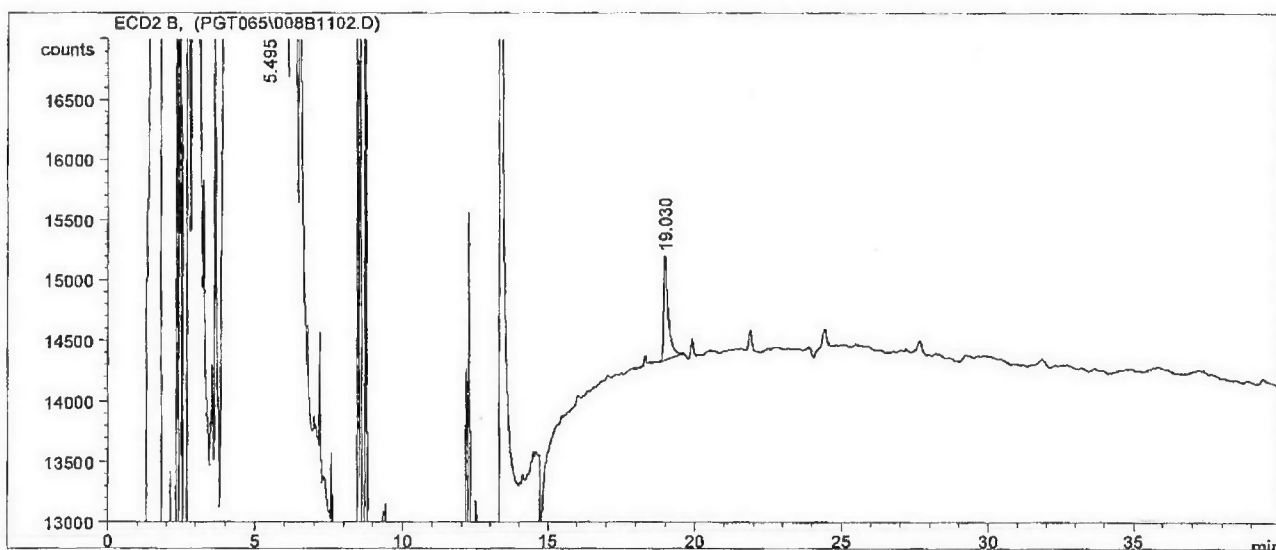


Typical Chromatography

Determination B, Sample 1

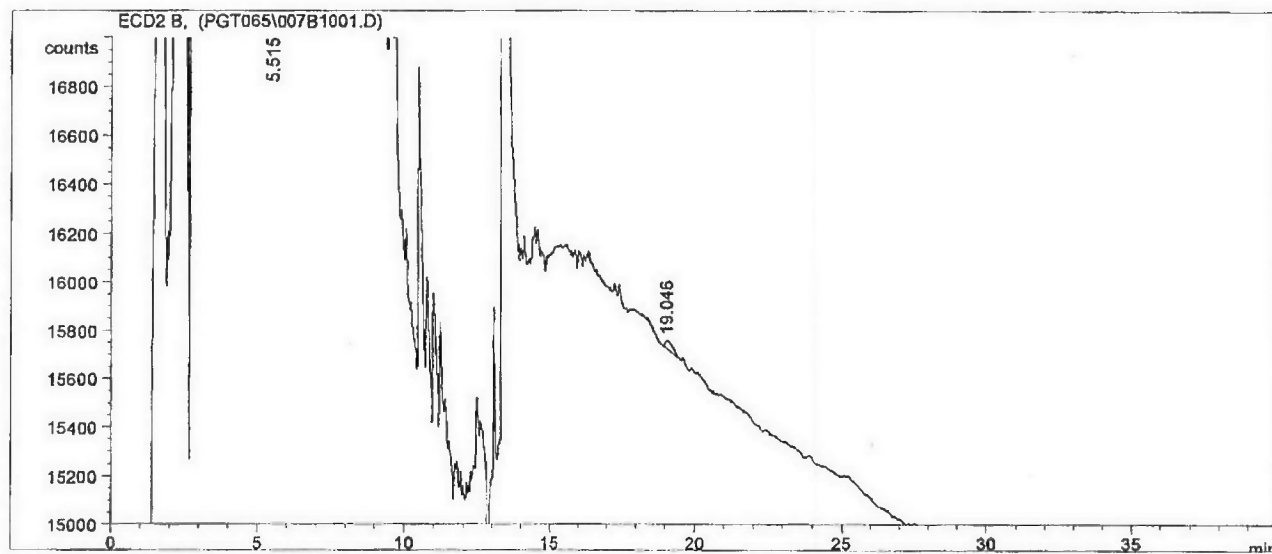


Determination C, Sample 7

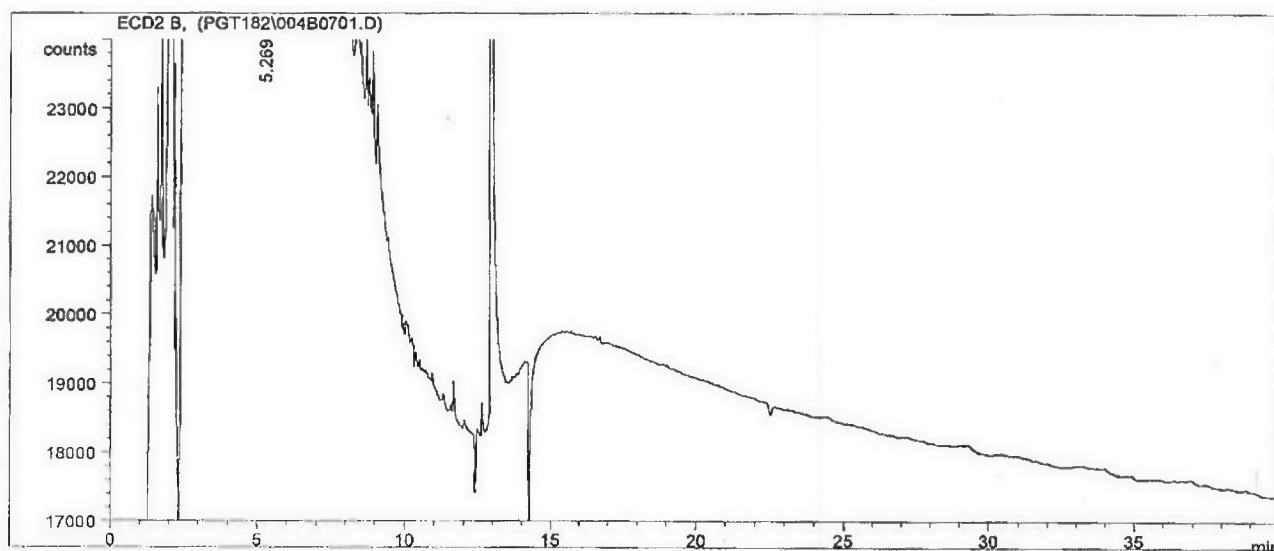


Typical Chromatography

Determination D, Sample 6



Matrix Blank



8.3 Calculation

The mean test material: internal standard peak area ratio of each standard was corrected to a nominal concentration of 10 mg/l and the mean value taken.

The sample solution concentration (g/l) was calculated using Equation 8.1.

Equation 8.1

$$C_{spl} = \frac{P_{spl}}{P_{std}} \times C_{std} \times D \times \frac{1}{1000}$$

where:

- C_{spl} = sample concentration (g/l)
- P_{spl} = mean test material: internal standard peak area ratio of sample solution
- P_{std} = mean test material: internal standard peak area ratio of standard solution, corrected to nominal standard concentration
- C_{std} = nominal standard concentration (10 mg/l)
- D = sample dilution factor (0.01)

8.4 Results

8.4.1 Preliminary test

The preliminary estimate of water solubility was less than 9.6×10^{-4} g/l.

8.4.2 Definitive test

The mean test material: internal standard peak area ratios relating to the standard and sample solutions are shown in the following tables:

Table 8.2

Solution	Mean peak area ratio
Standard 9.70 mg/l	0.218
Standard 10.1 mg/l	0.249
Sample A1	2.075×10^{-3}
Sample B1	7.364×10^{-4}
Sample A2	1.688×10^{-3}
Sample 2B	7.177×10^{-4}
Standard 9.70 mg/l	0.211
Standard 10.1 mg/l	0.212
Sample A3	2.085×10^{-3}
Sample B3	3.545×10^{-4}
Sample A4	7.472×10^{-3}
Sample B4	3.347×10^{-4}
Sample A5	3.289×10^{-3}
Sample B5	8.317×10^{-4}
Standard 9.70 mg/l	0.287
Standard 10.1 mg/l	0.361
Sample A6	6.382×10^{-3}
Sample B6	7.325×10^{-4}
Standard 9.70 mg/l	0.229
Standard 10.1 mg/l	0.274
Sample A7	1.142×10^{-2}
Sample A8	1.604×10^{-3}

Table 8.3

Solution	Mean peak area ratio
Standard 10.0 mg/l	0.213
Standard 10.1 mg/l	0.219
Sample C1	6.998×10^{-4}
Sample D1	3.220×10^{-4}
Standard 10.0 mg/l	0.321
Standard 10.1 mg/l	0.371
Sample C2	4.599×10^{-4}
Sample D2	2.587×10^{-4}
Standard 10.0 mg/l	0.262
Standard 10.1 mg/l	0.364
Sample C3	2.454×10^{-4}
Sample D3	2.881×10^{-2}
Standard 10.0 mg/l	0.335
Standard 10.1 mg/l	0.340
Sample C4	4.128×10^{-4}
Sample D4	1.495×10^{-4}
Sample C5	2.909×10^{-4}
Sample D5	9.436×10^{-5}
Standard 10.0 mg/l	0.278
Standard 10.1 mg/l	0.343
Sample C6	2.662×10^{-4}
Sample D6	1.476×10^{-4}
Sample C7	2.742×10^{-3}
Sample D7	3.835×10^{-4}

The concentration (g/l) of test material in the sample solutions are shown in the following tables:

Table 8.4

Sample Number	Sampling Time (hour)*		Concentration (g/l)		Solution pH	
	Determination A	Determination B	Determination A	Determination B	Determination A	Determination B
1	169¾	170¾	8.80×10^{-7}	3.13×10^{-7}	5.7	6.0
2	194¼	195¼	7.16×10^{-7}	3.05×10^{-7}	6.9	6.8
3	264¼	265¼	9.75×10^{-7}	1.66×10^{-7}	5.5	5.8
4	290¾	292	3.49×10^{-6}	1.57×10^{-7}	5.6	5.7
5	313¾	314¼	1.54×10^{-6}	3.89×10^{-7}	5.8	5.6
6	337¾	338¾	1.96×10^{-6}	2.24×10^{-7}	5.7	5.8
7	456¼	†	4.51×10^{-6}	†	5.3	†
8	483¼	†	6.33×10^{-7}	†	5.5	†

Table 8.5

Sample Number	Sampling Time (hour)*		Concentration (g/l)		Solution pH	
	Determination C	Determination D	Determination C	Determination D	Determination C	Determination D
1	307	307¼	3.25×10^{-7}	1.50×10^{-7}	5.7	5.7
2	333½	333¾	1.34×10^{-7}	7.52×10^{-8}	5.3	5.5
3	427¾	428	7.88×10^{-8}	9.25×10^{-6}	6.1	6.0
4	451½	451¾	1.23×10^{-7}	4.45×10^{-8}	6.0	5.8
5	474	474½	8.66×10^{-8}	2.81×10^{-8}	5.5	5.6
6	502¼	502¾	8.63×10^{-8}	4.78×10^{-8}	5.8	5.8
7	570¼	570½	8.89×10^{-7}	1.24×10^{-7}	6.1	5.3

* To the nearest ¼ hour

† Sample lost during preparation.

A correction for recovery of analysis of 21.2% was applied (see discussion section for further information). The results are shown in the following table:

Table 8.6

Determination	Maximum Concentration (g/l)	Maximum Concentration Corrected for Recovery (g/l)
A	4.51×10^{-6}	$<2.12 \times 10^{-5}$
B	3.89×10^{-7}	$<1.83 \times 10^{-6}$
C	8.89×10^{-7}	$<4.19 \times 10^{-6}$
D	9.25×10^{-6}	$<4.36 \times 10^{-5}$
Overall	9.25×10^{-6}	$<4.36 \times 10^{-5}$

Atom/Fragment Contribution

Using compositional data supplied by the Sponsor, the water solubility of the test material has been estimated to be less than 3.24×10^{-9} g/l at 25°C (WSKOWWIN v1.41) and less than 4.43×10^{-7} g/l at 25°C (WATERNT v1.01).

8.5 Validation

The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 0 to 15 mg/l. This was satisfactory with a correlation coefficient of 1.00 being obtained.

Recovery of analysis of the sample procedure was assessed and proved adequate for the test.

At a nominal concentration of 0.005 mg/l, a mean percentage recovery of 21.2% was obtained (range 16.8 to 28.9%).

Overall concentrations have been corrected for recovery of analysis.

8.6 Discussion

Based on the results obtained from systems A and B, evidence of a trend was observed in that the solubility in water increased with a decrease in flow rate. Further determinations were therefore performed (systems C and D) to investigate this further. Collating all the results together it was considered that no trend existed and that the differences in the results were due to analytical variation only at these extremely low concentrations. Due to this variability, it was considered more appropriate to quote the overall result as a less than the highest concentration observed in the samples to obtain a worst-case result.

Using compositional data supplied by the Sponsor, the water solubility of the test material has been estimated to be less than 3.24×10^{-9} g/l at 25°C (WSKOWWIN v1.41) and less than 4.43×10^{-7} g/l at 25°C (WATERNT v1.01).

8.7 Conclusion

The water solubility of the test material has been determined to be less than 4.36×10^{-5} g/l of solution at $20.0 \pm 0.5^\circ\text{C}$.

9. PARTITION COEFFICIENT

9.1 Method

The determination was carried out using the HPLC Method, Method A8 Partition Coefficient of Commission Regulation (EC) No 440/2008 of 30 May 2008.

9.2 Procedure

9.2.1 Preliminary estimate

A preliminary assessment of the partition coefficient was made based on the approximate solubilities of the test material in n-octanol and water. This was carried out by visual assessment.

9.2.2 Definitive test

Preparation of sample solution

Test material (0.0488 g) was diluted to 100 ml with tetrahydrofuran.

Preparation of dead time solution

The dead time was determined by measuring the retention time of thiourea (purity* >99.0 %, 17 mg/l solution in methanol).

Preparation of reference standard solution

A solution of reference standards (see following table) was prepared in methanol.

Table 9.1

Standard	Purity (%)*	Concentration (mg/l)
Benzene	99.98	5.00×10^3
Toluene	≥ 98.0	3.28×10^3
Naphthalene	≥ 99.0	423
Phenanthrene	≥ 97.0	21
Triphenylamine	98.0	195
DDT	98.0	166

* Value quoted by supplier

Determination of retention time

The sample, dead time and reference standard solutions were injected in duplicate using the following HPLC parameters:

HPLC System	: Agilent Technologies 1100, incorporating workstation
Column	: Eclipse XDB-C8 5µm (250 x 4.6 mm id)
Column temperature	: 40°C
Mobile phase	: methanol: glass double-distilled water (75:25 v/v)*
Typical pH of mobile phase	: 6.6
Flow-rate	: 1.0 ml/min
Injection volume	: 25 µl
UV detector wavelength	: 240 nm

Construction of calibration curve

A calibration curve was constructed from the retention time data of the dead time and reference standard solutions (Figure 9.1). The capacity factors (k) for the reference standards were calculated using Equation 9.2. $\log_{10} P_{ow}$ values of the reference standards are those quoted in Method 117 of the OECD Guidelines for Testing of Chemicals, 13 April 2004.

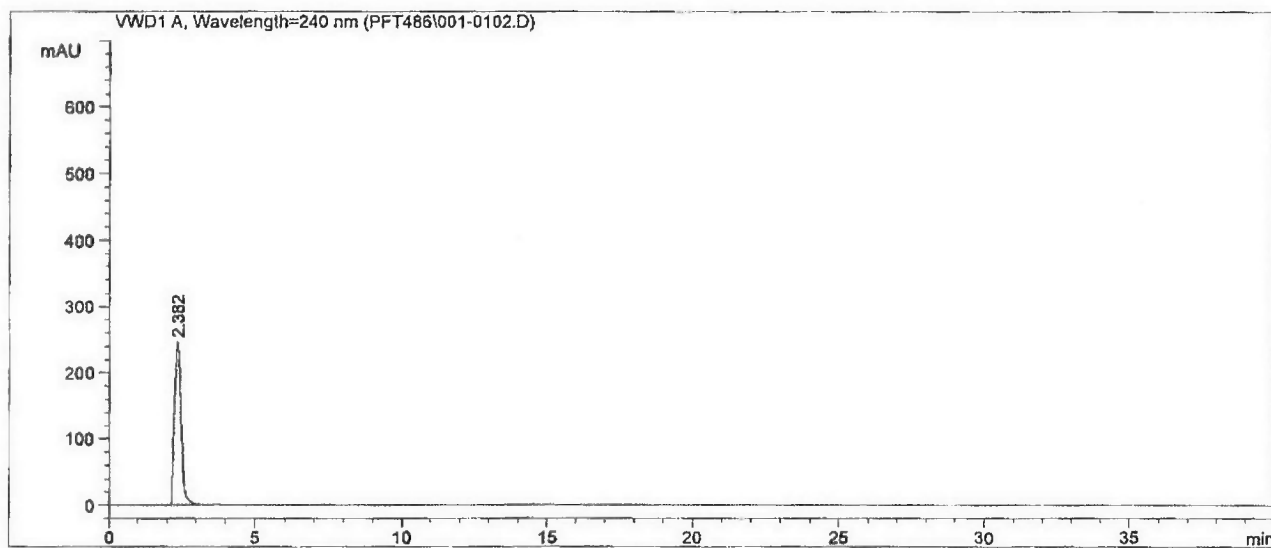
Partition coefficient of sample

The capacity factor was calculated using Equation 9.2 and the $\log_{10} P_{ow}$ value determined using Equation 9.3 with reference to the calibration curve (Figure 9.1).

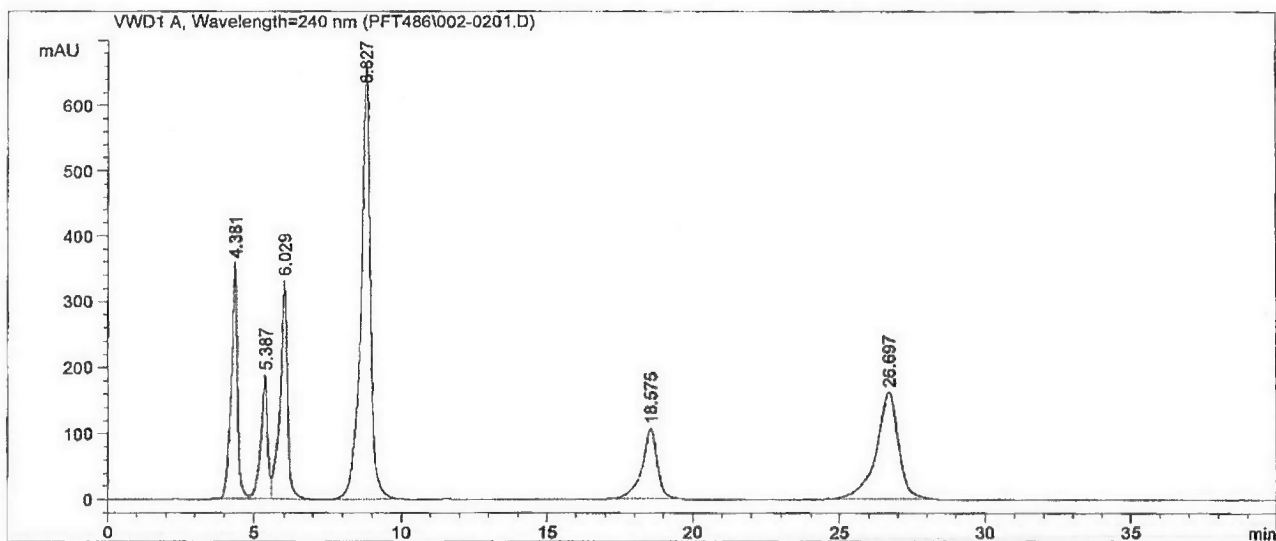
* A gradient elution comprising of a change of the mobile phase to 97% methanol after the retention time of DDT was utilised to elute the highly retained test material components remaining on the HPLC column.

Typical Chromatography

Dead Time

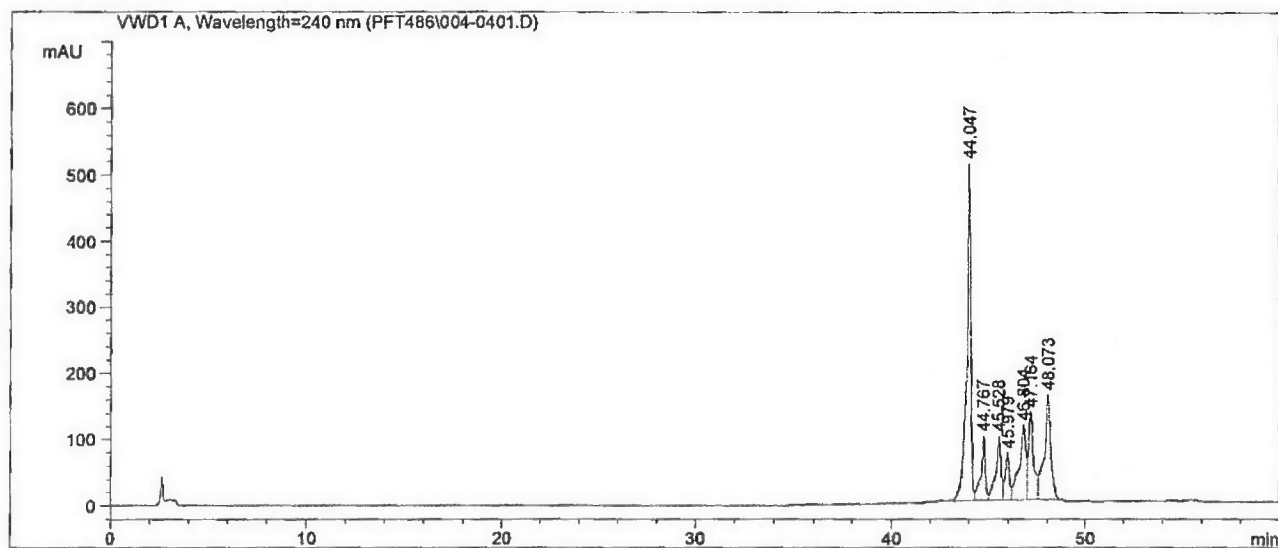


Reference Standards



Typical Chromatography

Sample



9.3 Calculations

9.3.1 Preliminary estimate

The preliminary estimate of the partition coefficient was calculated using Equation 9.1.

Equation 9.1

$$P_{ow} \text{ estimate} = \frac{\text{solubility of the test material in n - octanol}}{\text{solubility of the test material in water}}$$

9.3.2 Capacity factor

The capacity factor was determined using 9.2.

Equation 9.2

$$k = \frac{t_r - t_0}{t_0}$$

where:

- k = capacity factor
- t_r = retention time (min)
- t_0 = dead time (min)

9.3.3 Partition Coefficient

The partition coefficient was calculated using Equation 9.3.

Equation 9.3

$$\text{Log}_{10} P_{ow} = \frac{\text{Log}_{10} k - A}{B}$$

where:

- P_{ow} = partition coefficient
- k = capacity factor
- A = intercept of the calibration curve (Figure 9.1)
- B = slope of the calibration curve (Figure 9.1)

9.4 Results

9.4.1 Preliminary estimate

Approximate solubility in n-octanol: $<1.01 \times 10^{-2}$ g/l

Approximate solubility in water: $<8.7 \times 10^{-3}$ g/l

The test material was observed to be essentially insoluble in both n-octanol and water, therefore an estimate of the partition coefficient by visual estimation was considered inaccurate and essentially meaningless.

9.4.2 Definitive test

Calibration

The retention times of the dead time and the retention times, capacity factors (k) and $\log_{10} P_{ow}$ values for the reference standards are shown in the following tables:

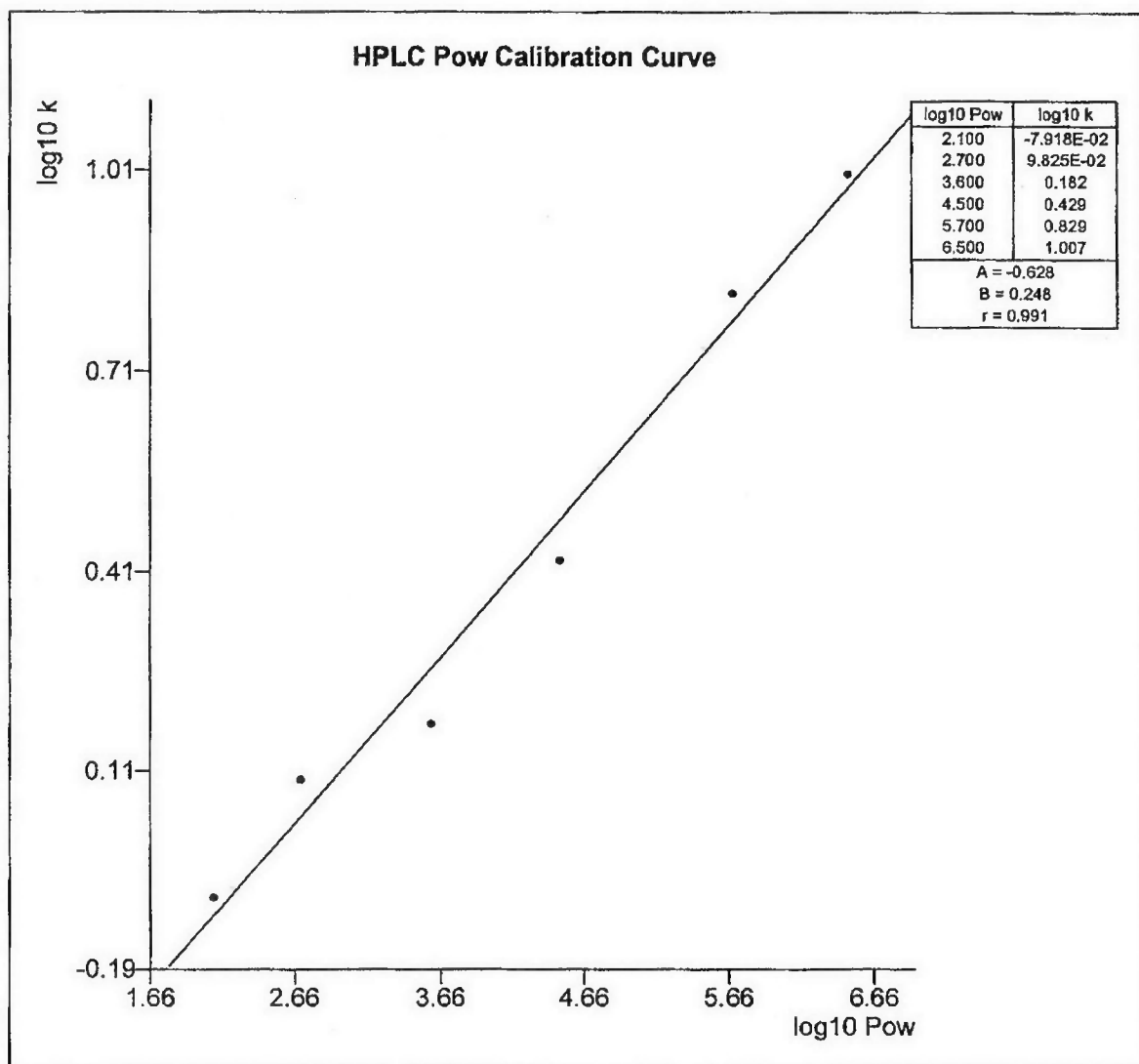
Table 9.2

Dead Time	Retention Time (mins)		Mean Retention Time (mins)
	Injection 1	Injection 2	
Thiourea	2.400	2.382	2.391

Table 9.3

Standard	Retention Time (mins)		Mean Retention Time (mins)	Capacity Factor (k)	$\log_{10} k$	$\log_{10} P_{ow}$
	Injection 1	Injection 2				
Benzene	4.381	4.386	4.384	0.833	-7.92×10^{-2}	2.1
Toluene	5.387	5.391	5.389	1.25	9.83×10^{-2}	2.7
Naphthalene	6.029	6.028	6.029	1.52	0.182	3.6
Phenanthrene	8.827	8.798	8.813	2.69	0.429	4.5
Triphenylamine	18.575	18.436	18.506	6.74	0.829	5.7
DDT	26.697	26.698	26.698	10.2	1.01	6.5

Figure 9.1 Calibration Curve



Partition coefficient of sample

The retention times, capacity factors and $\log_{10} P_{ow}$ values determined for the sample are shown in the following table:

Table 9.4

Injection	Retention Time (mins)	Capacity Factor (k)	$\log_{10} k$	$\log_{10} P_{ow}$
1	>26.698	>10.2	>1.01	>6.5
2	>26.698	>10.2	>1.01	>6.5

Mean $\log_{10} P_{ow}$: >6.5

Partition coefficient: $>3.16 \times 10^6$

9.5 Discussion

Substances having a $\log_{10} P_{ow}$ greater than 3 are regarded as having the potential to bioaccumulate in the environment.

As the test material was considered to contain no dissociation constants, testing was carried without pH adjustment to the mobile phase.

Only one peak (the earliest eluting) was used in the determination of the partition coefficient. This was due to all peaks eluting after the retention time of DDT. Results for all peaks would be >6.5.

9.6 Conclusion

The partition coefficient of the test material has been determined to be greater than 3.16×10^6 , $\log_{10} P_{ow} > 6.5$.

10. PARTICLE SIZE DISTRIBUTION

10.1 Method

The method used is designed to comply with that given in 'Particle Size Distribution, Fibre Length and Diameter Distribution', June 1996 European Commission technical guidance document, which satisfies the requirements of OECD Guideline 110.

10.2 Procedure

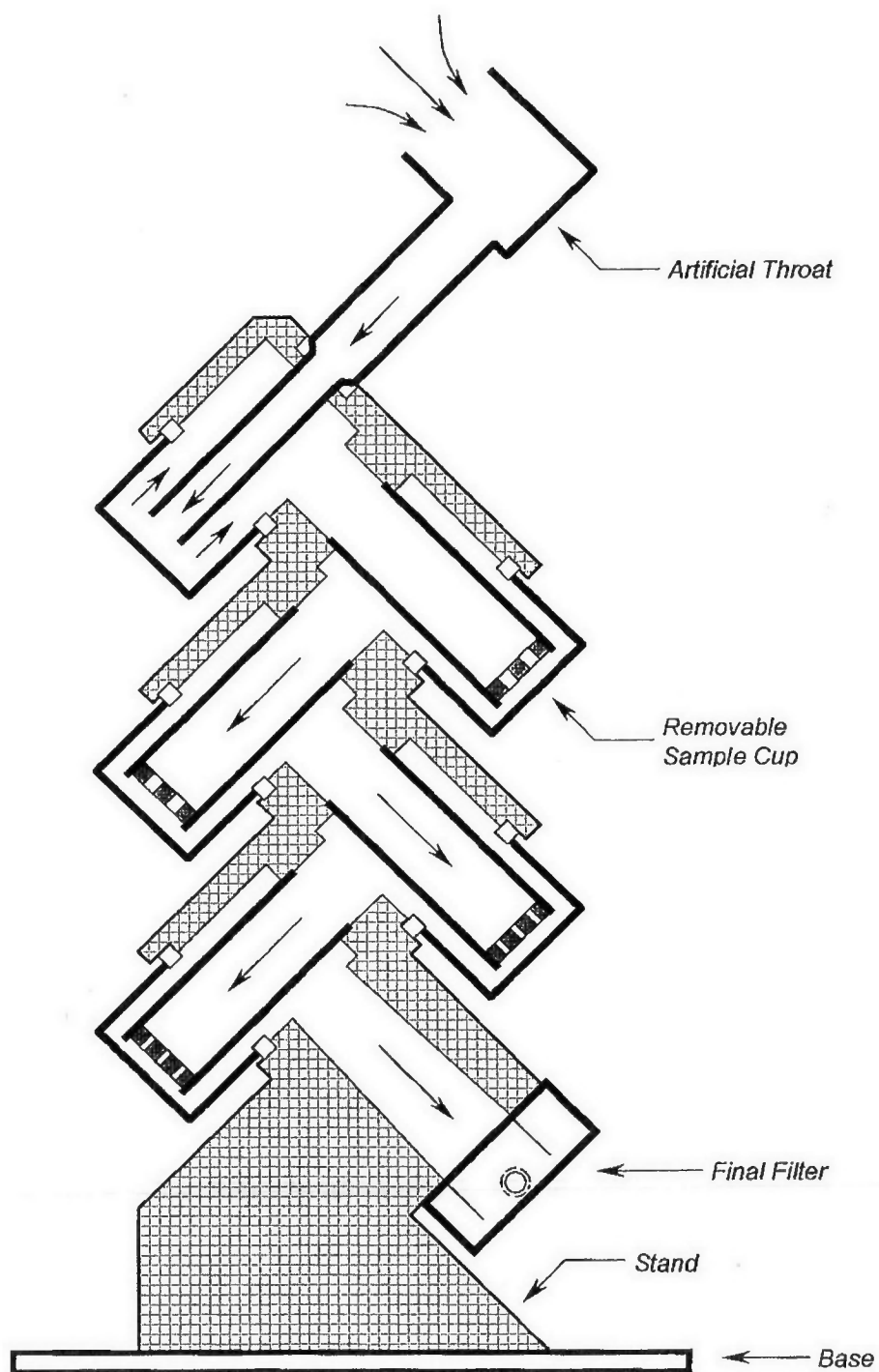
10.2.1 Screening test (sieve method)

Test material (15.16 g) was sieved for approximately 30 minutes on an Inclyno sieve shaker using a 100 μm sieve. The mass of test material passing through the sieve was measured.

10.2.2 Definitive test (cascade impactor method)

Test material (approximately 3g) was passed into the inlet port of a Marple Miller Cascade Impactor (MSP Corporation, Minneapolis, Minnesota, USA - Figure 10.1). The cascade impactor operates at a flow rate of 60 litres/minute and make-up atmospheric air was allowed to enter the impactor around the inlet port. The impactor consists of five stages with cut-point aerodynamic diameters of 10.0, 5.5, 2.4, 1.61 and 0.307 μm , and a final glass fibre filter. Particles not deposited in the artificial throat are deposited, according to size, onto the bottom of the collection cups. The collection cups, artificial throat, and final filter were weighed before and after the run and the weight of test material collected at each stage calculated by difference.

Figure 10.1 Cascade Impactor



10.3 Calculations

Using the values obtained from the cascade impactor, the cumulative mass and cumulative % were calculated as shown in the following table:

Table 10.1

Collection Site		Mass at Stage (g)	Cumulative Mass (g)	Cumulative (%)
Entry Port	(0)	m_0	M_0	100
10.0 μm stage	(1)	m_1	M_1	$100 \times M_1/M_0$
5.5 μm stage	(2)	m_2	M_2	$100 \times M_2/M_0$
2.4 μm stage	(3)	m_3	M_3	$100 \times M_3/M_0$
1.61 μm stage	(4)	m_4	M_4	$100 \times M_4/M_0$
0.307 μm stage	(5)	m_5	M_5	$100 \times M_5/M_0$
Filter	(6)	m_6	M_6	$100 \times M_6/M_0$

where:

m = mass of material found at indicated site (g)

M = cumulative mass (g) at indicated site (e.g. $M_3 = m_3 + m_4 + m_5 + m_6$)

10.4 Results

10.4.1 Screening test (sieve method)

The results of the sieving procedure are shown in the following table:

Table 10.2

Sieve aperture size (μm)	100
Mass of test material transferred to sieve (g)	15.16
Mass of test material passed through sieve (g)	2.89
Test material less than sieve aperture size (%)	19.1

10.4.2 Definitive test (cascade impactor method)

Determination 1

The results of the cascade impactor method are shown in the following table:

Table 10.3

Cup Number	Particle Size Range Collected (μm)	Cup Masses (g)		
		Pre-sampling	Post-sampling	Difference
1	>10.0	86.1232	88.7975	2.6743
2	5.5 - 10.0	85.3980	85.4401	0.0421
3	2.4 - 5.5	85.9180	85.9238	0.0058
4	1.61 - 2.4	85.6326	85.6351	0.0025
5	0.307 - 1.61	85.7975	85.7994	0.0019
Filter	<0.307	75.0919	75.0948	0.0029

Amount of test material found in artificial throat: 0.20 g.

Total amount of test material recovered from impactor cups, filter and artificial throat: 2.93 g.

The cumulative amounts found for the individual particle size cut-points (μm) are shown in the following table:

Table 10.4

Cup Number	Particle Size Cut-points (μm)	Cumulative Mass of Test Material (g)	Cumulative Amount of Test Material (%)
2	10.0	0.0552	1.88
3	5.5	0.0131	0.447
4	2.4	0.0073	0.249
5	1.61	0.0048	0.164
Filter	0.307	0.0029	9.90×10^{-2}

Determination 2

The results of the cascade impactor method are shown in the following table:

Table 10.5

Cup Number	Particle Size Range Collected (μm)	Cup Masses (g)		
		Pre-sampling	Post-sampling	Difference
1	>10.0	86.1224	89.1552	3.0328
2	5.5 - 10.0	85.3977	85.4047	0.0070
3	2.4 - 5.5	85.9168	85.9179	0.0011
4	1.61 - 2.4	85.6336	85.6347	0.0011
5	0.307 - 1.61	85.7957	85.7960	0.0003
Filter	<0.307	75.0886	75.0896	0.0010

Amount of test material found in artificial throat: 0.14 g.

Total amount of test material recovered from impactor cups, filter and artificial throat:
3.18 g.

The cumulative amounts found for the individual particle size cut-points (μm) are shown in the following table:

Table 10.6

Cup Number	Particle Size Cut-points (μm)	Cumulative Mass of Test Material (g)	Cumulative Amount of Test Material (%)
2	10.0	0.0105	0.330
3	5.5	0.0035	0.110
4	2.4	0.0024	7.54×10^{-2}
5	1.61	0.0013	4.08×10^{-2}
Filter	0.307	0.0010	3.14×10^{-2}

Determination 3

The results of the cascade impactor method are shown in the following table:

Table 10.7

Cup Number	Particle Size Range Collected (μm)	Cup Masses (g)		
		Pre-sampling	Post-sampling	Difference
1	>10.0	86.1408	89.0326	2.8918
2	5.5 - 10.0	85.4161	85.4260	0.0099
3	2.4 - 5.5	85.9196	85.9211	0.0015
4	1.61 - 2.4	85.6535	85.6580	0.0045
5	0.307 - 1.61	85.7993	85.7998	0.0005
Filter	<0.307	75.0885	75.0889	0.0004

Amount of test material found in artificial throat: 0.07 g.

Total amount of test material recovered from impactor cups, filter and artificial throat: 2.98 g.

The cumulative amounts found for the individual particle size cut-points (μm) are shown in the following table:

Table 10.8

Cup Number	Particle Size Cut-points (μm)	Cumulative Mass of Test Material (g)	Cumulative Amount of Test Material (%)
2	10.0	0.0168	0.564
3	5.5	0.0069	0.232
4	2.4	0.0054	0.181
5	1.61	0.0009	3.02×10^{-2}
Filter	0.307	0.0004	1.34×10^{-2}

The overall cumulative amounts of test material with a particle size less than 10.0 μm (%) from Determinations 1 to 3 are shown in the following table:

Table 10.9

Determination	Cumulative Amount of Test Material Less Than 10.0 μm (%)	Mean Cumulative Amount of Test Material Less Than 10.0 μm (%)
1	1.88	0.926
2	0.330	
3	0.564	

The overall cumulative amounts of test material with a particle size less than 5.5 μm (%) from Determinations 1 to 3 are shown in the following table:

Table 10.10

Determination	Cumulative Amount of Test Material Less Than 5.5 μm (%)	Overall Cumulative Amount of Test Material Less Than 5.5 μm (%)
1	0.447	0.263
2	0.110	
3	0.232	

10.5 Discussion

Too few particles were of a size less than 10.0 μm to allow accurate assessment of mass median aerodynamic diameter.

Sampling was performed by rolling the sample container for approximately 10 minutes, and sampling from the top, middle and bottom prior to definitive testing. Some inconsistencies in the results were observed indicating that the sample was not homogenous. Since the samples were taken from three different areas, the overall mean results can be considered representative of the particle size distribution for the test material.

The inhalable fraction is defined as the mass fraction of particles which can be inhaled by nose or mouth, the thoracic fraction is defined as the mass fraction of particles that passes the larynx and the respirable fraction is defined as the mass fraction of particles that reaches the alveoli.

10.6 Conclusion

Particle size data acquired for the test material are shown in the following table:

Table 10.11

Measurement	Method	Result
Proportion of test material having an inhalable particle size less than 100 μm	Sieve	19.1 %
Proportion of test material having a thoracic particle size less than 10.0 μm	Cascade Impactor	0.926 %
Proportion of test material having a respirable particle size less than 5.5 μm	Cascade Impactor	0.263 %

Appendix 1 Statement of GLP Compliance in accordance with Directive 2004/9/EC

**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM****GOOD LABORATORY PRACTICE****STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC****TEST FACILITY**

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE


Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


4/3/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority


MHRA

**DETERMINATION OF HAZARDOUS
PHYSICO-CHEMICAL PROPERTIES**

PROJECT NUMBER: 2737/0005

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QUALITY ASSURANCE REPORT

This study type is classed as short-term. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§ 09 February 2009	Protocol Compliance Audit
09 March 2009	Vapour Pressure
03,12 February 2009	Flammability (Solids)
10,11 march 2009	Relative self-Ignition Temperature for Solids
03,04 February 2009	Moisture Content
§ 28 April 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit

§ Evaluation specific to this study



DATE: 19 JUN 2009

For the Quality Assurance Unit*

***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

With the exception of explosive and oxidising properties predictions the work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

..... *S. S. Atwal* DATE: 1.9 JUN 2009

S S Atwal BSc (Hons)

STUDY DIRECTOR

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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DETERMINATION OF HAZARDOUS PHYSICO-CHEMICAL PROPERTIES

SUMMARY

Hazardous physico-chemical properties were determined using methods specified in Commission Regulation (EC) No 440/2008 of 30 May 2008.

Test	Method	Result
Vapour Pressure	A4	Less than 4.5×10^{-4} Pa at 25°C
Flammability (Solids)	A10	Not highly flammable
Explosive Properties	A14	Predicted negative
Relative Self-Ignition Temperature for Solids	A16	None below the melting temperature
Oxidising Properties	A17	Predicted negative

DETERMINATION OF HAZARDOUS PHYSICO-CHEMICAL PROPERTIES

1. INTRODUCTION

Hazardous physico-chemical properties of the test material have been determined. Methods employed were those specified in Commission Regulation (EC) No 440/2008 of 30 May 2008, Part A: Methods for the determination of physico-chemical properties.

Testing was conducted between 19 February 2009 and 08 April 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	[]
Description	:	white powder
Batch number	:	20090105
Date received	:	23 January 2009
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

3. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

4. VAPOUR PRESSURE

4.1 Method

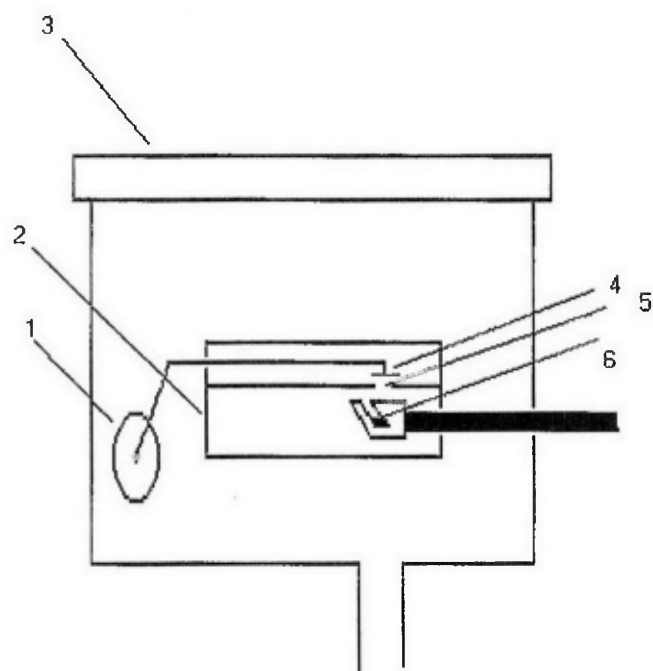
The vapour pressure was determined using a vapour pressure balance with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure at 25°C. Testing was conducted using Method A4 Vapour Pressure of Council Regulation (EC) No 440/2008 of 30 May 2008.

4.2 Procedure

The vapour pressure was determined using a vapour pressure balance. The temperature of the sample was controlled electronically. The mass and temperature readings were recorded automatically into a computer file.

A diagram of the cross-section of the vapour pressure balance is represented in Figure 4.1. After evacuating the system, opening the shutter above the sample oven causes the escaping vapour jet to be directed at the scale pan. The difference in mass readings with the orifice covered and uncovered is proportional to the vapour pressure at the given oven temperature.

A sequence of runs was started after a sample of test material had been under vacuum for approximately 69½ hours. Temperature and pressure readings were taken between 120 and 130 °C with a one hour dwell at 120 °C between runs.

Figure 4.1 Schematic Diagram of the Apparatus Used

- 1 Microbalance
- 2 Oven
- 3 Glass viewing panel
- 4 Balance pan
- 5 Shutter with orifice
- 6 Test sample

4.3 Calculation

The vapour pressure is related to the observed mass difference by Equation 4.1.

Equation 4.1

$$V_p = \frac{\delta m \cdot g}{A}$$

where:

V_p	=	vapour pressure (Pa)
δm	=	mass difference (kg)
g	=	acceleration due to gravity (9.813 m s^{-2})
A	=	area of the orifice ($7.06858 \times 10^{-6} \text{ m}^2$)

Vapour pressure is related to temperature by Equation 4.2.

Equation 4.2

$$\text{Log}_{10} [V_p (\text{Pa})] = \frac{\text{slope}}{\text{temperature (K)}} + \text{intercept}$$

A plot of $\text{Log}_{10} V_p (\text{Pa})$ versus reciprocal temperature ($1/T(\text{K})$) therefore gives a straight line graph.

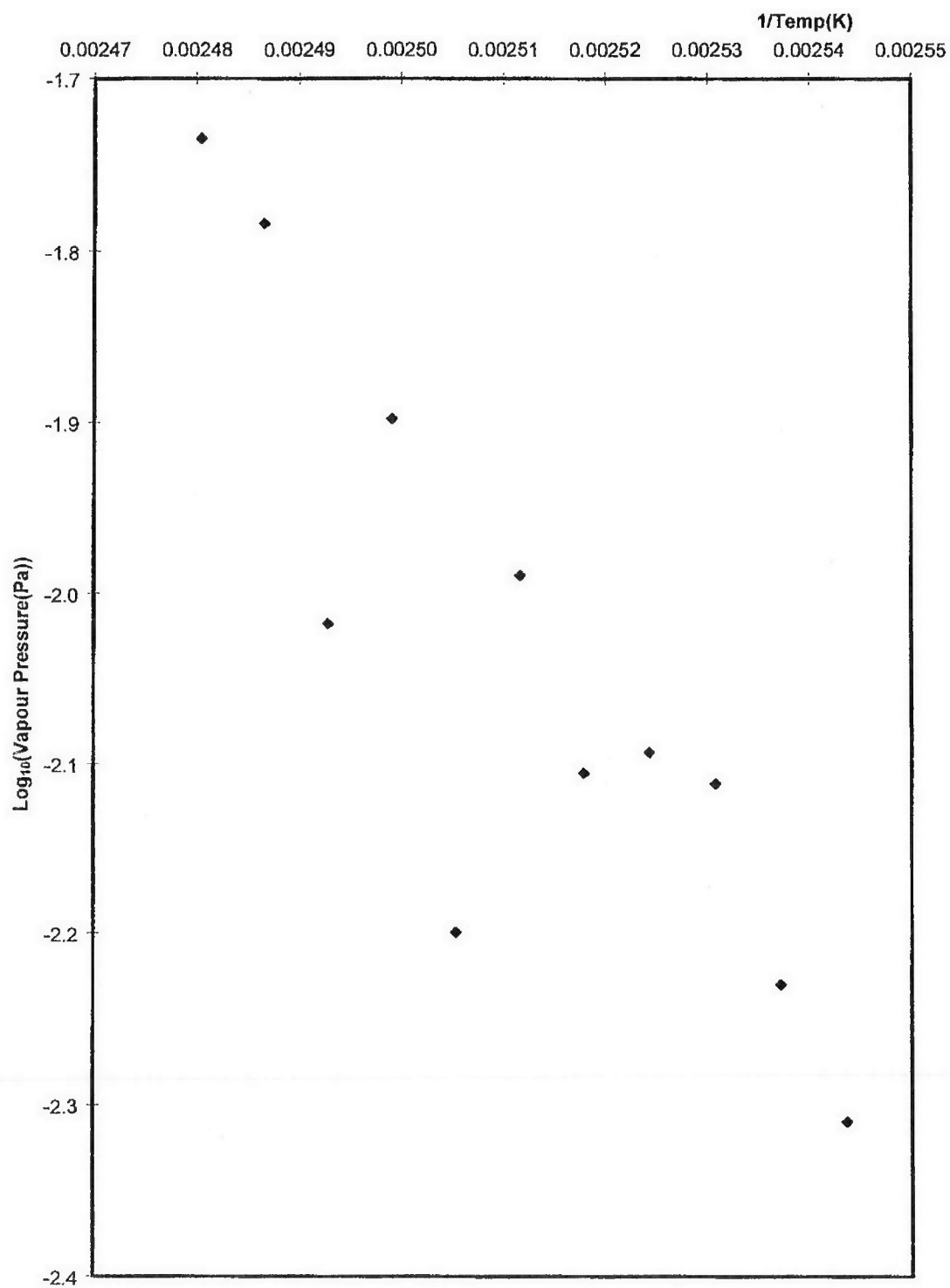
The vapour pressure of the sample was measured over a range of temperatures to enable extrapolation to 298.15 K.

4.4 Results

Run 1

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (μg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
120	393.15	0.002543558	3.53	3.530E-09	0.004900544	-2.309755673
121	394.15	0.002537105	4.24	4.240E-09	0.005886206	-2.230164622
122	395.15	0.002530685	5.57	5.570E-09	0.007732587	-2.111675184
123	396.15	0.002524296	5.81	5.810E-09	0.008065769	-2.093354246
124	397.15	0.002517940	5.65	5.650E-09	0.007843648	-2.105481931
125	398.15	0.002511616	7.38	7.380E-09	0.010245331	-1.989474017
126	399.15	0.002505324	4.55	4.550E-09	0.006316566	-2.199518982
127	400.15	0.002499063	9.11	9.110E-09	0.012647014	-1.898012002
128	401.15	0.002492833	6.91	6.910E-09	0.009592850	-2.018052331
129	402.15	0.002486634	11.85	1.185E-08	0.016450836	-1.783812028
130	403.15	0.002480466	13.27	1.327E-08	0.018422160	-1.734659456

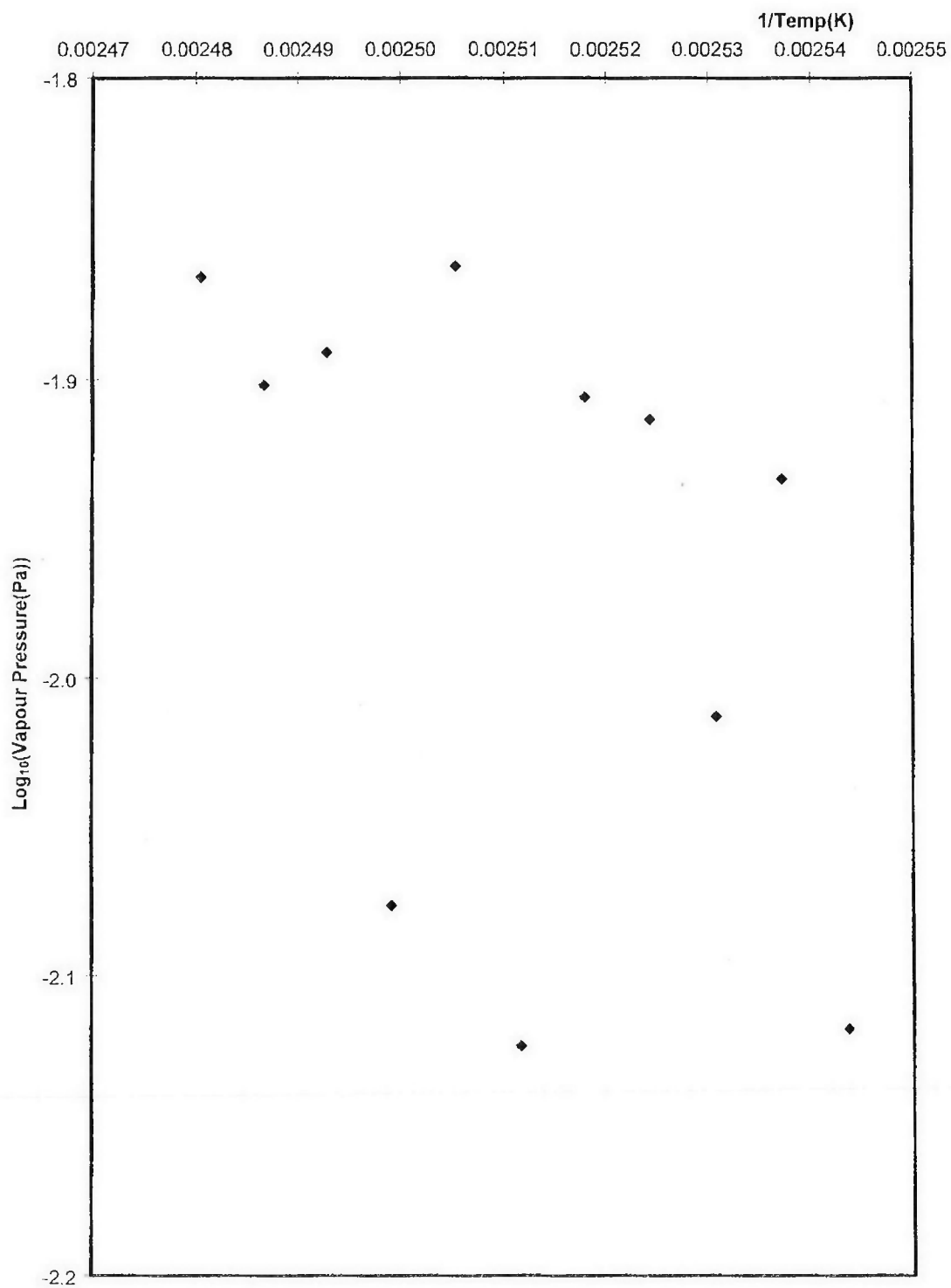
No statistical analysis is given due to the nature of the plot.

Run 1 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

Run 2

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
120	393.15	0.002543558	5.49	5.490E-09	0.007621527	-2.117958034
121	394.15	0.002537105	8.40	8.400E-09	0.011661352	-1.933251093
122	395.15	0.002530685	6.99	6.990E-09	0.009703911	-2.013053203
123	396.15	0.002524296	8.79	8.790E-09	0.012202772	-1.913541504
124	397.15	0.002517940	8.95	8.950E-09	0.012424893	-1.905707343
125	398.15	0.002511616	5.42	5.420E-09	0.007524349	-2.123531092
126	399.15	0.002505324	9.89	9.890E-09	0.013729854	-1.862334087
127	400.15	0.002499063	6.04	6.040E-09	0.008385067	-2.076493440
128	401.15	0.002492833	9.26	9.260E-09	0.012855252	-1.890919392
129	402.15	0.002486634	9.03	9.030E-09	0.012535953	-1.901842628
130	403.15	0.002480466	9.81	9.810E-09	0.013618793	-1.865861371

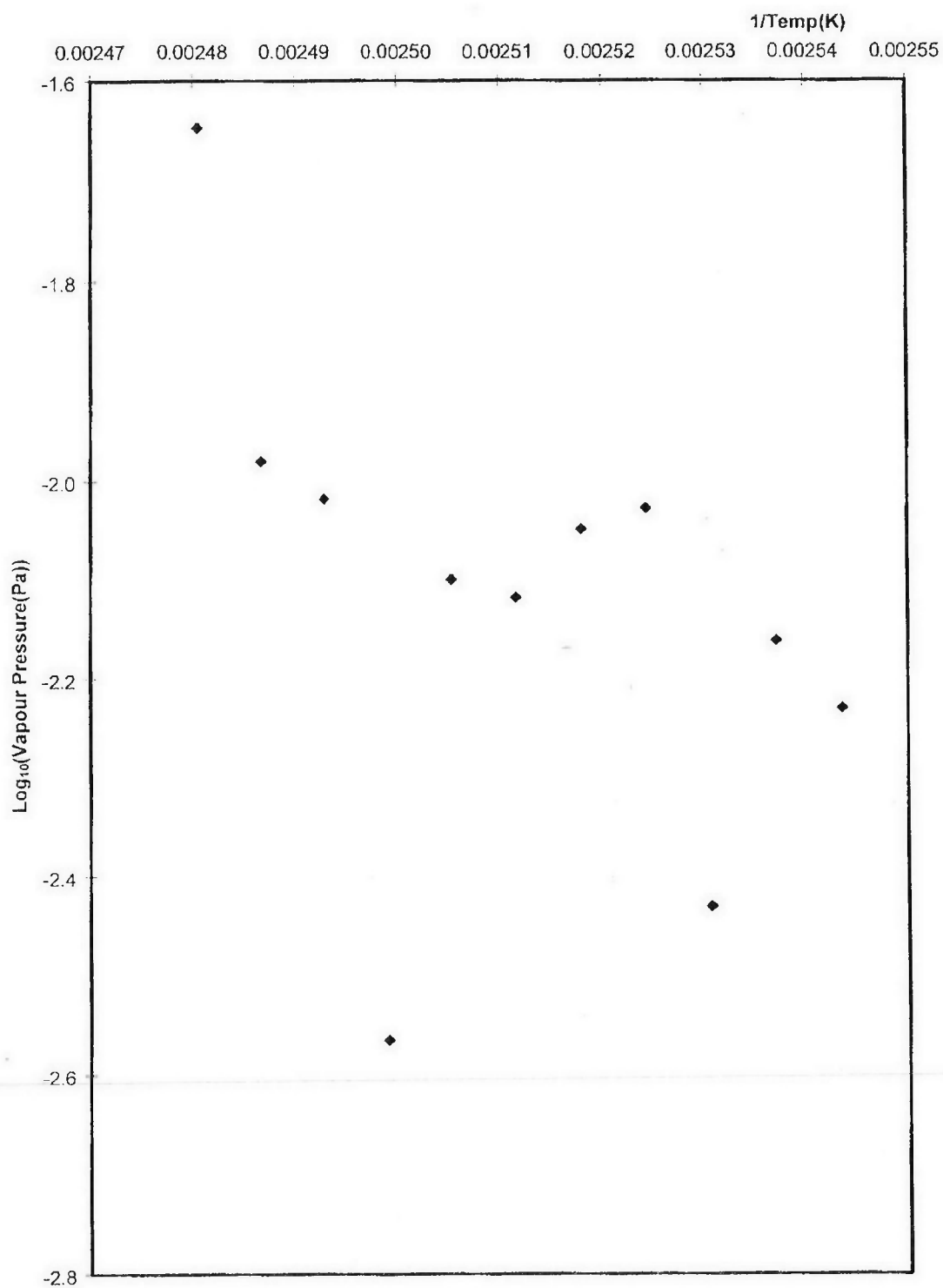
No statistical analysis is given due to the nature of the plot.

Run 2 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

Run 3

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
120	393.15	0.002543558	4.24	4.240E-09	0.005886206	-2.230164522
121	394.15	0.002537105	4.95	4.950E-09	0.006871868	-2.162925180
122	395.15	0.002530685	2.67	2.670E-09	0.003706644	-2.431019117
123	396.15	0.002524296	6.75	6.750E-09	0.009370729	-2.028226606
124	397.15	0.002517940	6.44	6.440E-09	0.008940370	-2.048644511
125	398.15	0.002511616	5.49	5.490E-09	0.007621527	-2.117958034
126	399.15	0.002505324	5.73	5.730E-09	0.007954708	-2.099375757
127	400.15	0.002499063	1.96	1.960E-09	0.002720982	-2.565274307
128	401.15	0.002492833	6.91	6.910E-09	0.009592850	-2.018052331
129	402.15	0.002486634	7.54	7.540E-09	0.010467452	-1.980159033
130	403.15	0.002480466	16.25	1.625E-08	0.022559163	-1.646677013

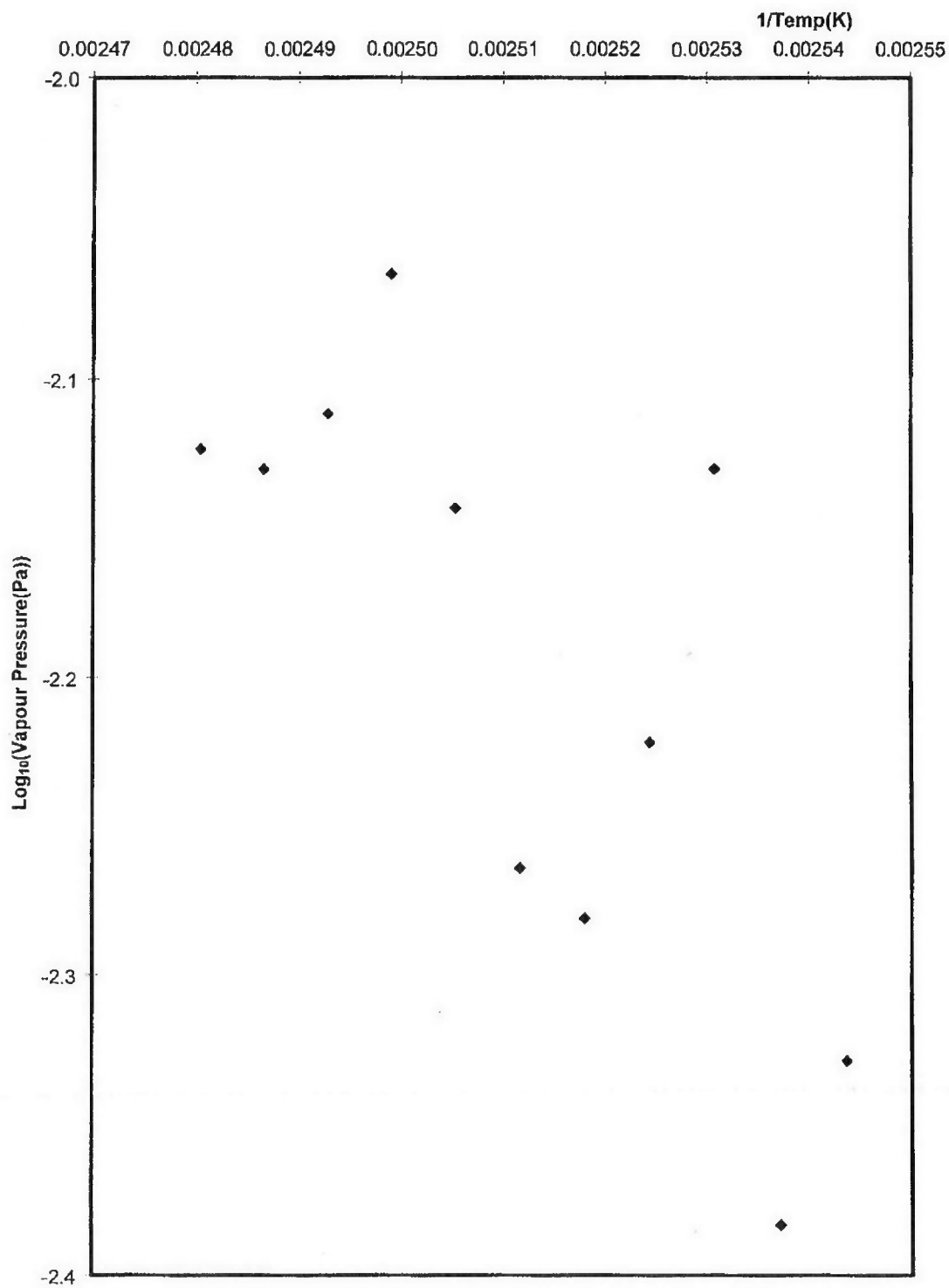
No statistical analysis is given due to the nature of the plot.

Run 3 - Graph of \log_{10} Vapour Pressure vs Reciprocal Temperature

Run 4

Temperature (°C)	Temperature (K)	Reciprocal Temperature (K ⁻¹)	Mass Difference (µg)	Mass Difference (kg)	Vapour Pressure (Pa)	Log ₁₀ Vp
120	393.15	0.002543558	3.38	3.380E-09	0.004692306	-2.328613678
121	394.15	0.002537105	2.98	2.980E-09	0.004137003	-2.383314115
122	395.15	0.002530685	5.34	5.340E-09	0.007413288	-2.129989122
123	396.15	0.002524296	4.32	4.320E-09	0.005997267	-2.222046632
124	397.15	0.002517940	3.77	3.770E-09	0.005233726	-2.281189029
125	398.15	0.002511616	3.92	3.920E-09	0.005441964	-2.264244312
126	399.15	0.002505324	5.18	5.180E-09	0.007191167	-2.143200619
127	400.15	0.002499063	6.20	6.200E-09	0.008607188	-2.065138689
128	401.15	0.002492833	5.57	5.570E-09	0.007732587	-2.111675184
129	402.15	0.002486634	5.34	5.340E-09	0.007413288	-2.129989122
130	403.15	0.002480466	5.42	5.420E-09	0.007524349	-2.123531092

No statistical analysis is given due to the nature of the plot.

Run 4 - Graph of Log_{10} Vapour Pressure vs Reciprocal Temperature

The test material did not change in appearance under the conditions used in the determination.

4.5 Discussion

No statistical analyses were performed because the balance readings were too low and variable for a line of best fit to have any meaning. Instead it was considered more appropriate to impose a regression slope on a chosen data point to provide an estimate of the maximum value for the vapour pressure at 25°C.

Run 4 was chosen because the sample had been under vacuum for the longest period prior to this run and so degassing would have been the most complete. The reading at 127°C (400.15 K) was chosen because this is the data point which gives the highest estimated vapour pressure at any given temperature when a slope of -1500 K is imposed upon it.

The value of -1500 K is an in-house value and is the shallowest slope observed whilst determining the vapour pressure on a wide range of samples using the vapour pressure balance method. Extrapolation to 25°C gave a vapour pressure of 4.492×10^{-4} Pa which has been taken as a maximum for this material.

4.6 Conclusion

The vapour pressure of the test material has been determined to be less than 4.5×10^{-4} Pa at 25°C.

5. FLAMMABILITY (SOLIDS)

5.1 Method

The flammability (solids) was determined by measuring the burning rate of test material prepared as a pile of set dimensions. Testing was conducted using Method A10 Flammability (Solids) of Commission Regulation (EC) No 440/2008 of 30 May 2008.

5.2 Procedures

5.2.1 Preliminary screening test

A mould (250 x 20 x 10 mm) was loosely filled with test material (tested as received). A non-combustible, non-porous board was placed onto the mould which was then inverted. The mould was removed and an air-rich Bunsen burner flame applied to one end of the pile for two minutes.

5.2.2 Moisture content

The moisture content was determined gravimetrically.

An aliquot (approximately 1 g) of test material was weighed (in duplicate; A and B) into loss bottles. The samples were dried to constant weight at approximately 105°C.

5.3 Calculation

The moisture content was calculated using Equation 5.1.

Equation 5.1

$$\text{Moisture Content (\%)} = \frac{b - c}{b - a} \times 100$$

Where:

- a = mass of loss bottle (g)
- b = mass of loss bottle and test material (g)
- c = mass of loss bottle and test material after drying (g)

5.4 Results

5.4.1 Preliminary screening test

The pile failed to ignite during the two minutes that the Bunsen flame was applied.

The result of the preliminary screening test obviated the need to perform the main test.

5.4.2 Moisture content

	Determination A	Determination B
a) Mass of loss bottle (g)	19.6938	19.4710
b) Mass of loss bottle and test material (g)	20.7005	20.5115
c) Mass of loss bottle and test material after drying (g)	20.7006	20.5115
Moisture content (% w/w)	<0.1%	<0.1%
Mean moisture content (% w/w)	<0.1%	

5.5 Conclusion

The test material has been determined to be not highly flammable as it failed to ignite in the preliminary screening test.

6. EXPLOSIVE PROPERTIES

6.1 Method

The explosive properties were predicted using Method A14 Explosive Properties of Commission Regulation (EC) No 440/2008 of 30 May 2008.

6.2 Procedure

The structure of the test material was assessed for chemical groups that imply explosive properties. Examples of such groups are C-C unsaturated, C-metal, N-metal, contiguous oxygen atoms, contiguous nitrogen atoms, N-halogens, O-halogens, N-O. Examples of these groups are given below.

Structural Features

C-C unsaturated

C-metal, N-metal

Contiguous oxygen atoms

Contiguous nitrogen atoms

N-halogens

O-halogens

N-O

Examples

Acetylenes, acetylides, 1,2 dienes

Grignard reagents, organo-lithium compounds

Peroxides, ozonides

Azides, aliphatic azo compounds, diazonium salts, hydrazines, sulphonyl hydrazides

Chloramines, fluoroamines

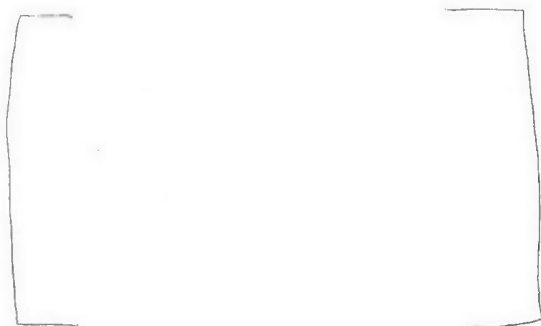
Chlorates, perchlorates, iodosyl compounds

Hydroxylamines, nitrates, nitro compounds, N-oxides, 1,2-oxazoles

Full structural details are given in Bretherick's Handbook of Reactive Chemical Hazards, 4th Edition, Butterworths, London, 1990.

6.3 Results

The test material is a mixture of the following compounds:



has the following structure:



The structures of the other compounds in the test material vary only by the number of



6.4 Conclusion

Based on the chemical structures of the components of the test material the result for the explosive properties has been predicted negative.

7. RELATIVE SELF-IGNITION TEMPERATURE FOR SOLIDS

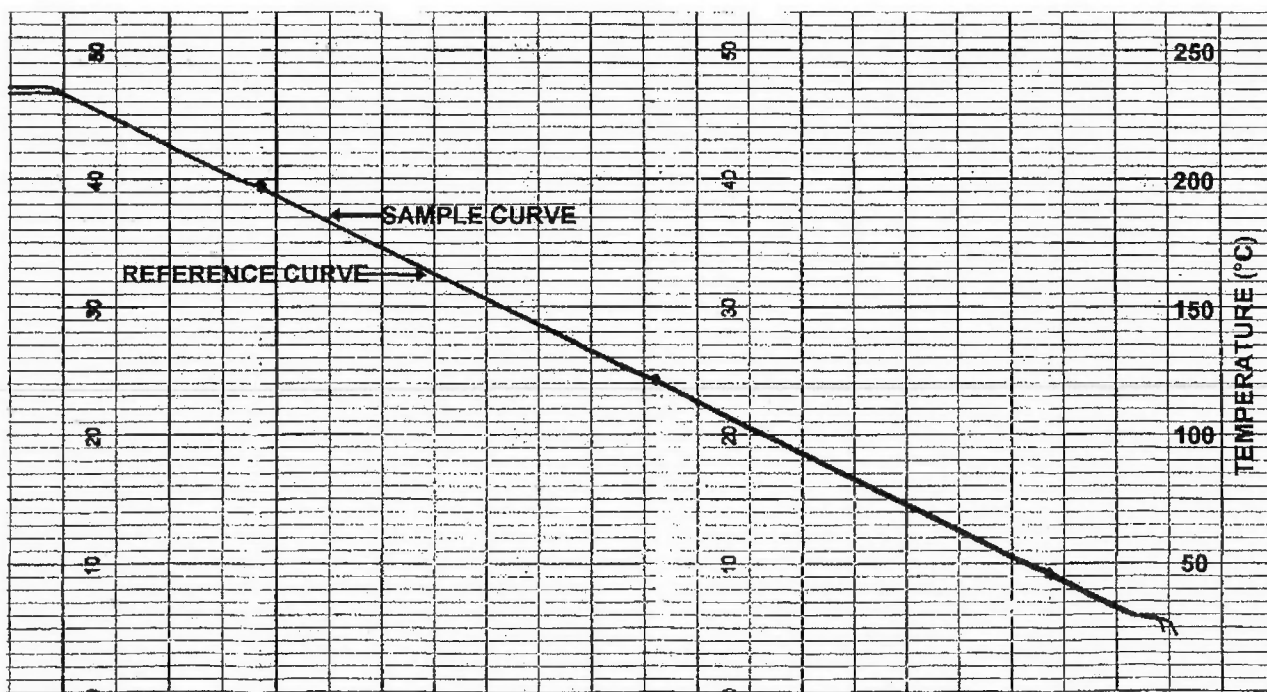
7.1 Method

The test material was heated in an oven and the relative self ignition temperature determined. Testing was conducted using Method A16 Relative Self-Ignition Temperature for Solids of Council Regulation (EC) No 440/2008 of 30 May 2008.

7.2 Procedure

An aliquot of the test material was suspended in a stainless steel mesh cube (approximately 20 x 20 x 20 mm) in an oven. A thermocouple was placed in the centre of the sample and another in the oven. The oven temperature was programmed to increase from ambient to 234°C (which was approximately 10°C higher than the maximum temperature of the melting range, determined as part of Harlan laboratories Ltd project number 2737/0004) at a rate of 0.5°C/min. The temperature/time curves relating to the condition in the centre of the sample and the oven were recorded on a two channel chart recorder.

7.3 Results



7.3.1 Observations after the test

The cube contained a small amount of a light brown fused solid residue on its base. A white crystalline solid residue was observed around the door and seals of the oven.

7.4 Conclusion

The test material has been determined not to have a relative self-ignition temperature below its melting temperature.

8. OXIDISING PROPERTIES (SOLIDS)/(LIQUIDS)

8.1 Method

The oxidising properties were predicted using Method A17 of Commission Regulation (EC) No 440/2008 of 30 May 2008.

8.2 Procedure

The structure of the test material was assessed for chemical groups that would imply oxidising properties. Examples of such groups are nitrates, metal oxides, hypofluorites, difluoroaminopolynitroaryls, perchlorates, bromates and iodites.

Full structural details are given in Bretherick's Handbook of Reactive Chemical Hazards, 4th Edition, Butterworths, London, 1990.

8.3 Results

The test material is a mixture of the following compounds:



has the following structure:



The structures of the other compounds in the test material vary only by the number of

8.4 Conclusion

Based on the chemical structures of the components of the test material the result for the oxidising properties has been predicted negative.

Appendix 1 Statement of GLP Compliance in accordance with Directive 2004/9/EC**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM****GOOD LABORATORY PRACTICE****STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC****TEST FACILITY**

Harlan Laboratories Ltd,
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

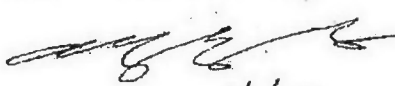
Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


4/3/09
Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



MHRA

DETERMINATION OF SPECTRA

PROJECT NUMBER: 2737/0006

AUTHORS: R E Butler
J A Walker
D F White

STUDY SPONSOR:

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(437-711) 332-2
Gocheon-dong
Uiwang-si
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KOREA

TEST FACILITY:

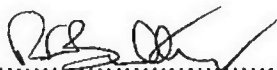
Harlan Laboratories Ltd
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AUTHENTICATION

I the undersigned, hereby declare that this report accurately reflects the original data generated in the study.



DATE: 10 JUL 2009

R E Butler GRSC
STUDY DIRECTOR

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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DETERMINATION OF SPECTRA

SUMMARY

Ultraviolet/visible absorption spectra. Under acidic, neutral, and alkaline conditions, the test material showed significant absorbance maxima below approximately 320 nm. Absorptivity data are shown in the following table:

Medium	Wavelength (nm)	Molar absorption coefficient ($\text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$)
Acidic (pH 1.7)	213.14	9.68×10^5
	281.69	1.44×10^3
	290.68	1.26×10^3
Neutral (pH 8.6)	212.95	8.74×10^5
	281.90	1.45×10^3
	290.74	1.27×10^3
Alkaline (pH 14.0)	213.55	7.15×10^5
	281.65	1.43×10^3
	290.84	1.26×10^3

Nuclear magnetic resonance spectra. The proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectra were consistent with the proposed chemical composition.

Infrared spectrum. The infrared spectrum was consistent with the proposed chemical composition.

DETERMINATION OF SPECTRA

1. INTRODUCTION

Spectra of the test material have been determined.

The proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectra were determined at the School of Chemistry, The University of Nottingham, University Park, Nottingham, NG7 2RD, UK.

Testing was conducted between 17 February 2009 and 02 April 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	
Description	:	white powder
Batch number	:	20090105
Date received	:	23 January 2009
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the purity and stability of the test material is the responsibility of the Sponsor. The identity of the test material has been addressed in this report.

2.2 Chemical Structure

The Sponsor provided the following chemical composition for the test material:



This composition was used for interpretation of the nuclear magnetic resonance and infrared spectra.

3. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

4. ULTRAVIOLET/VISIBLE ABSORPTION SPECTRA

4.1 Method

The determination was carried out using the procedure specified in Method 101 of the OECD Guidelines for Testing of Chemicals, 12 May 1981.

4.2 Procedure

4.2.1 Solution Preparation

Solutions (see following table) of test material were prepared in tetrahydrofuran (THF):

Table 4.1 - Solution Preparation

Medium	Matrix	Concentration	
		g/l	mol.dm ⁻³
Acidic	THF: 0.1M hydrochloric acid (90:10 v/v)	1.04×10^{-3}	1.43×10^{-6}
		0.753	1.03×10^{-3}
Neutral	THF: water (90:10 v/v)	1.04×10^{-3}	1.04×10^{-3}
		0.753	1.03×10^{-3}
Alkaline	THF: 0.1M sodium hydroxide (90:10 v/v)	1.04×10^{-3}	1.04×10^{-3}
		0.753	1.03×10^{-3}

4.2.2 Analysis

The test solutions were scanned using their respective matrices (Table 4.1) as the reference media.

The analysis parameters were as follows:

Spectrophotometer : Perkin-Elmer Lambda 20 double-beam spectrophotometer
Wavelength range : 190 - 900 nm
Cell path length : 1 cm
Cell type : Quartz

4.2.3 Calculation

The molar absorption coefficients (ϵ) were calculated using Equation 4.1.

Equation 4.1

$$\epsilon = \frac{A}{cl}$$

where:

A = absorbance
c = concentration (mol.dm^{-3})
l = cell path length (1 cm)

The molarity of the test solutions was calculated using the molecular weight of 734.5 g.mol^{-1} supplied by the Sponsor.

4.3 Results

4.3.1 Acidic Media

Figure 4.1 - Ultraviolet/Visible Absorption Spectrum in Acidic Media

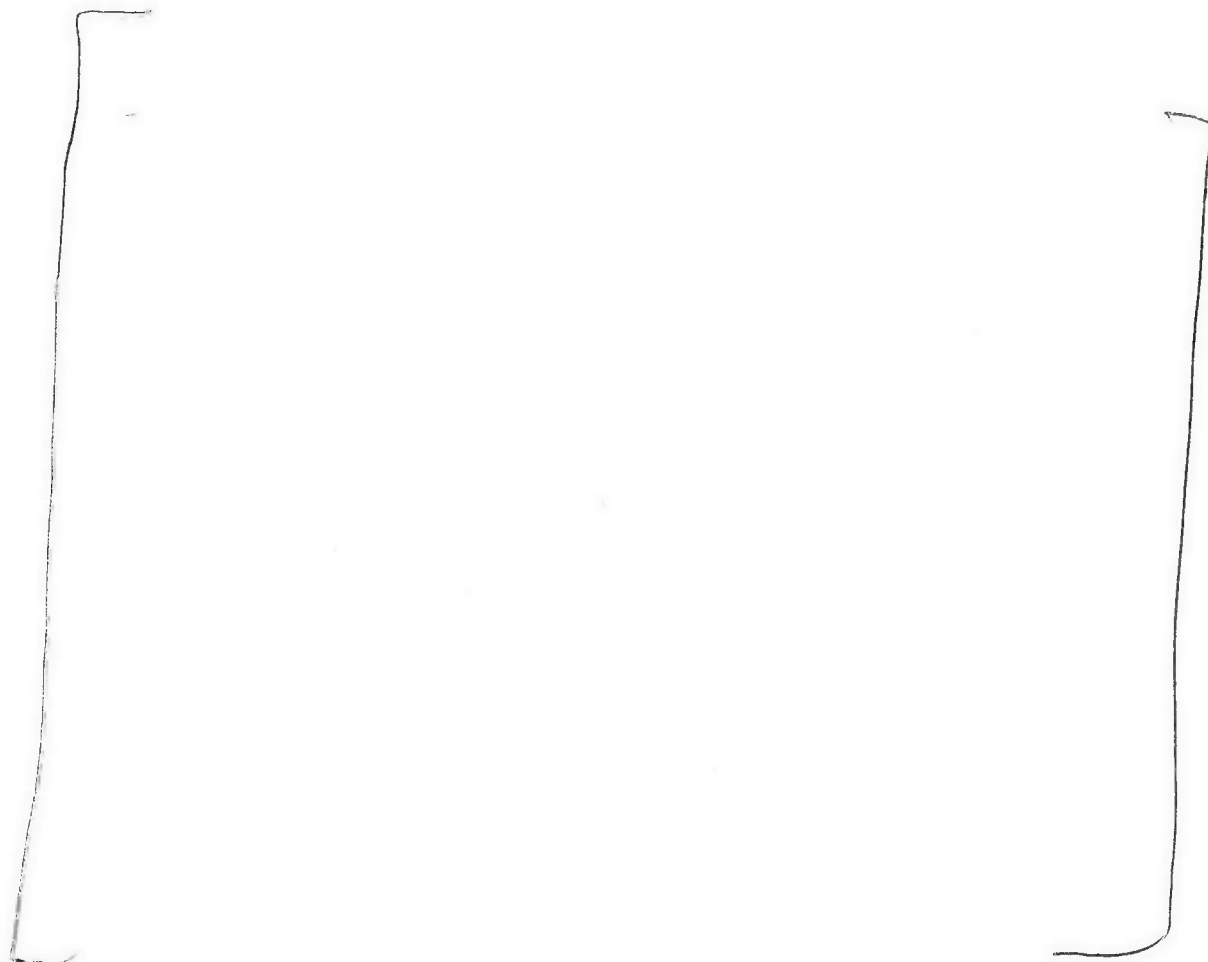
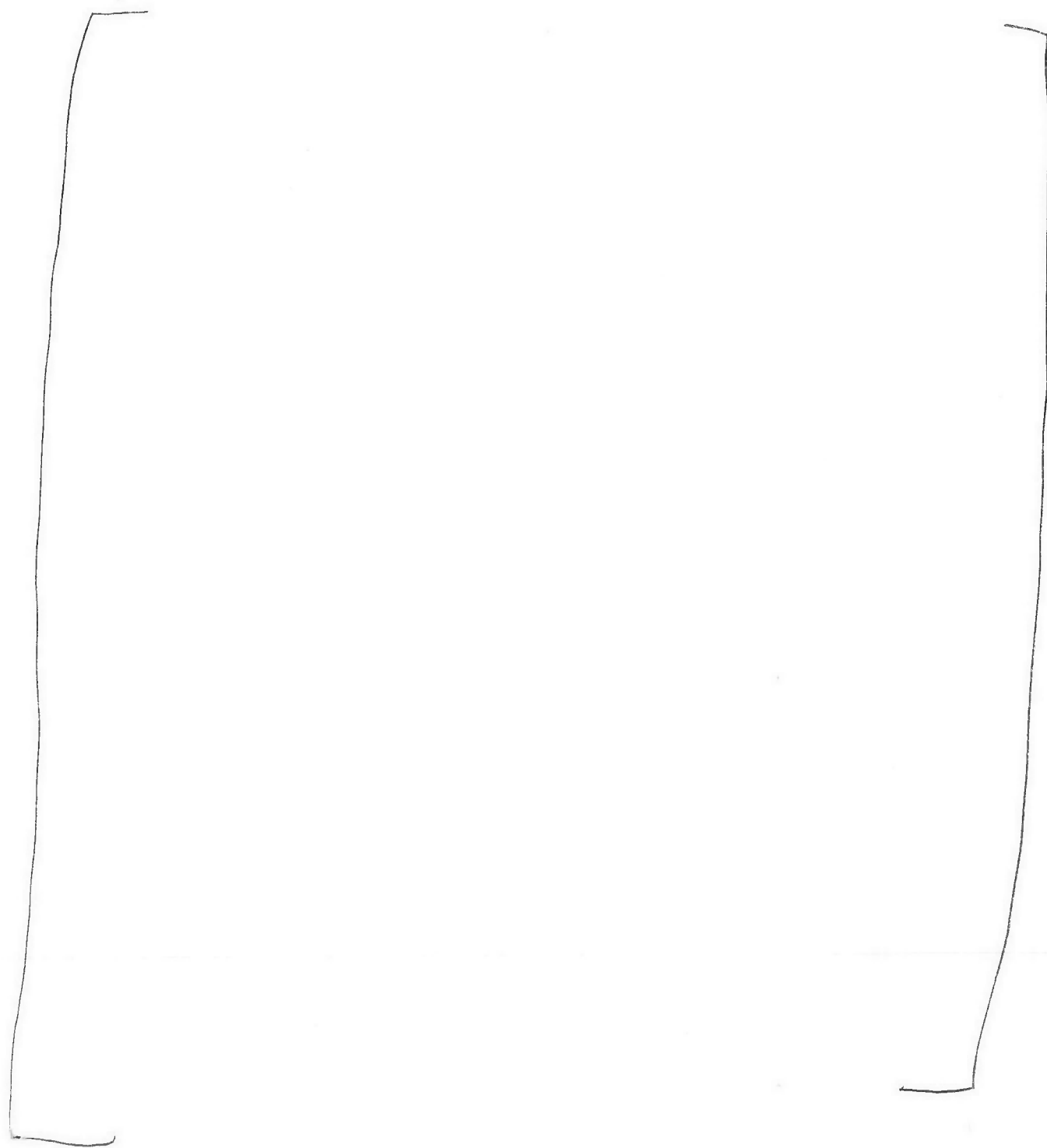


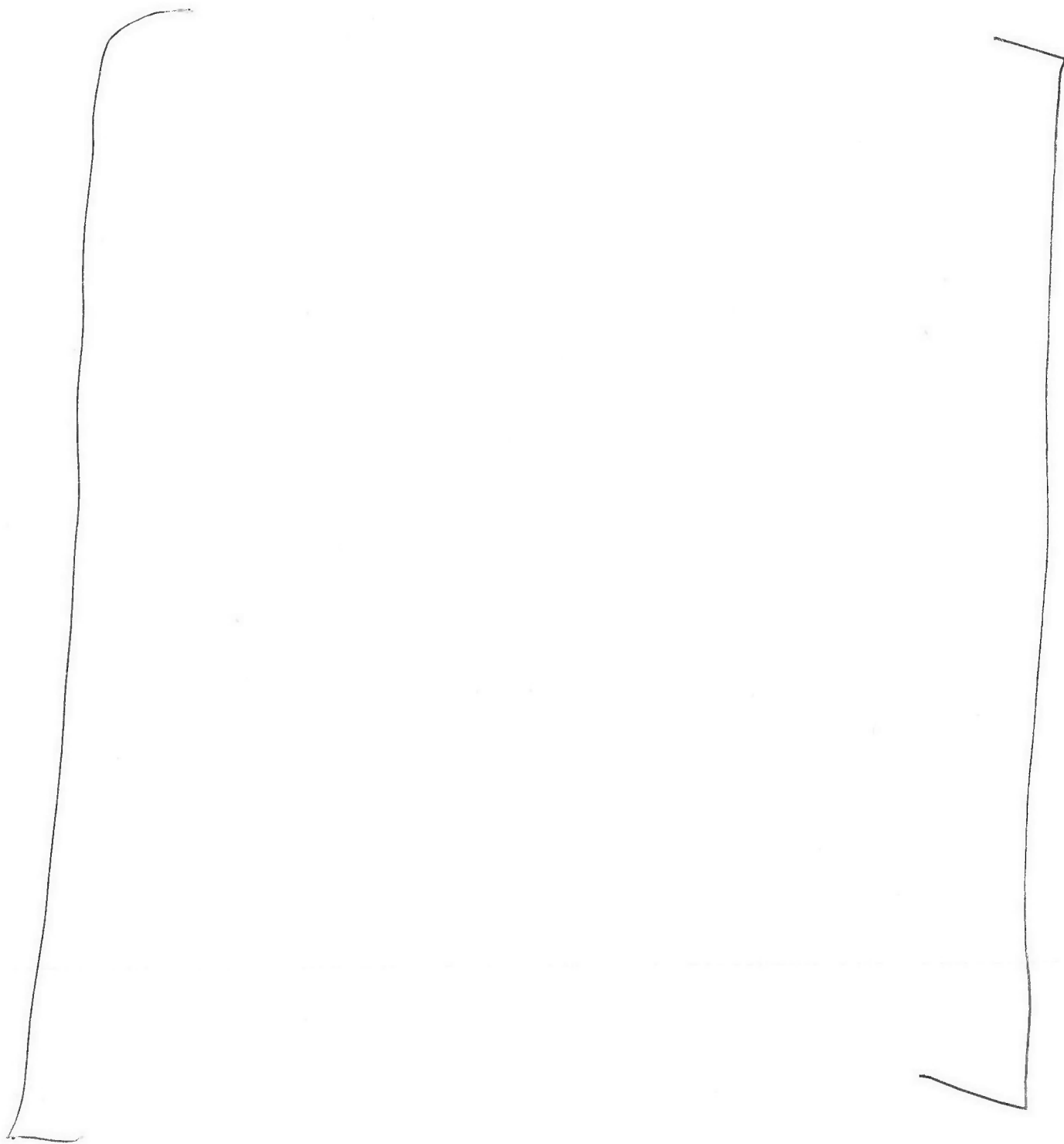
Figure 4.2 - Ultraviolet/Visible Absorption Spectrum in Acidic Media

4.3.2 Neutral Media

Figure 4.3 - Ultraviolet/Visible Absorption Spectrum in Neutral Media



Figure 4.4 - Ultraviolet/Visible Absorption Spectrum in Neutral Media

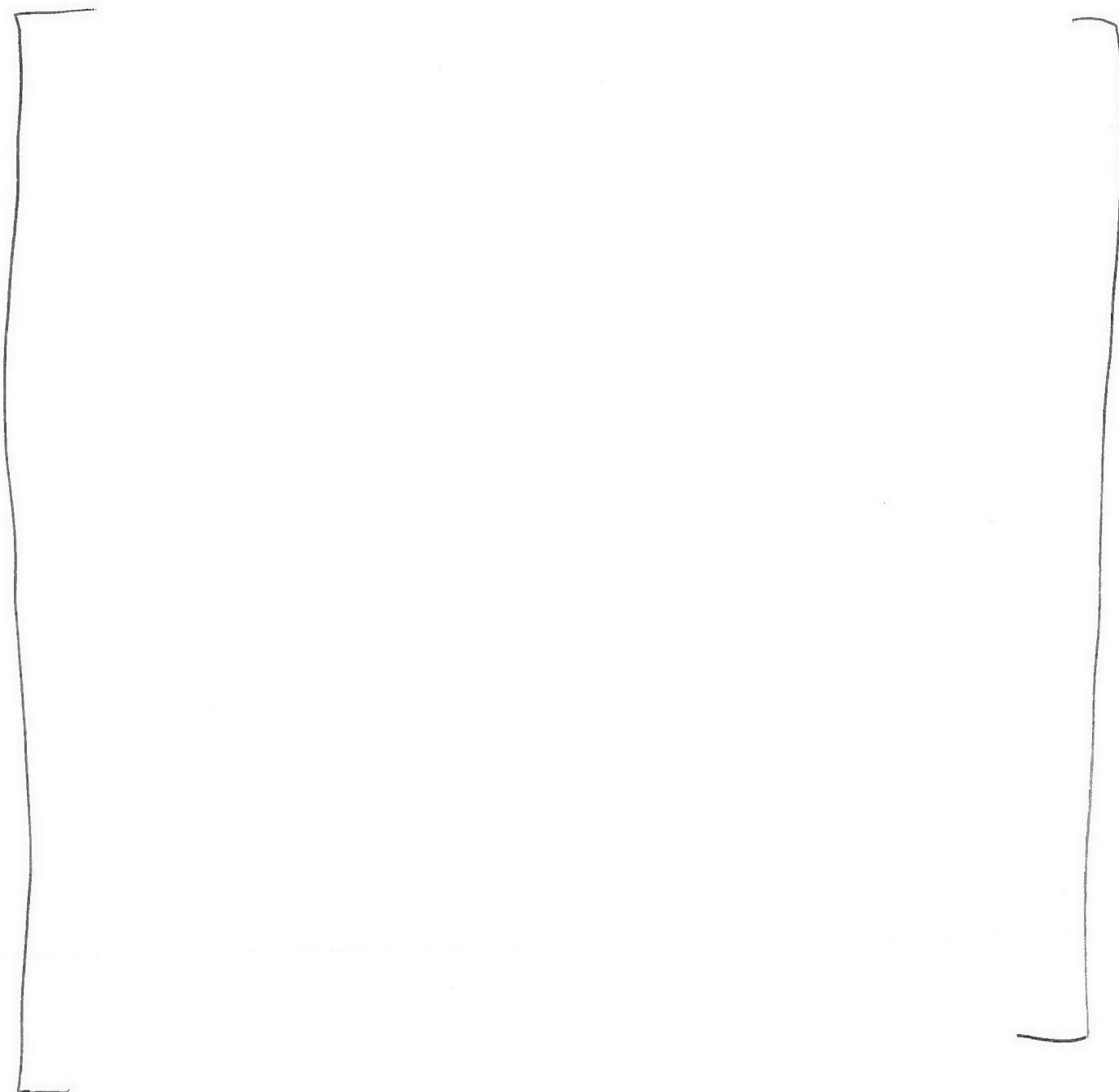


4.3.3 Alkaline Media

Figure 4.5 - Ultraviolet/Visible Absorption Spectrum in Alkaline Media



Figure 4.6 - Ultraviolet/Visible Absorption Spectrum in Alkaline Media



4.4 Conclusion

Under acidic, neutral, and alkaline conditions, the test material showed significant absorbance maxima below approximately 320 nm. Absorptivity data are shown in the following table:

Table 4.5



5. NUCLEAR MAGNETIC RESONANCE SPECTRA

5.1 Method

The proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were determined using a Brüker AV (III) 500 NMR spectrometer equipped with a dual proton/carbon cryoprobe.

5.2 Procedure

A proton (^1H) NMR spectrum of an approximately saturated solution of test material in deuterated tetrahydrofuran was obtained using the following parameters:

Nucleus	:	^1H
Operating temperature	:	ambient
Frequency	:	500 MHz
Reference	:	indirect calibration with tetramethylsilane

A carbon (^{13}C) NMR spectrum of an approximately saturated solution of test material in deuterated tetrahydrofuran was obtained using the following parameters:

Nucleus	:	^{13}C
Operating temperature	:	ambient
Frequency	:	126 MHz
Reference	:	indirect calibration with tetramethylsilane

An additional sub-spectra was also acquired using a Distortionless Enhancement through Polarisation Transfer (DEPT) technique. The preliminary use of the technique was to identify C-H signals. The instrument program DEPT 135 gave CH and CH_3 signals as positive peaks and CH_2 signals as negative peaks. No response was obtained for carbons associated with no protons, such as ester carbonyls and substituted aromatic carbons.

5.3 Results

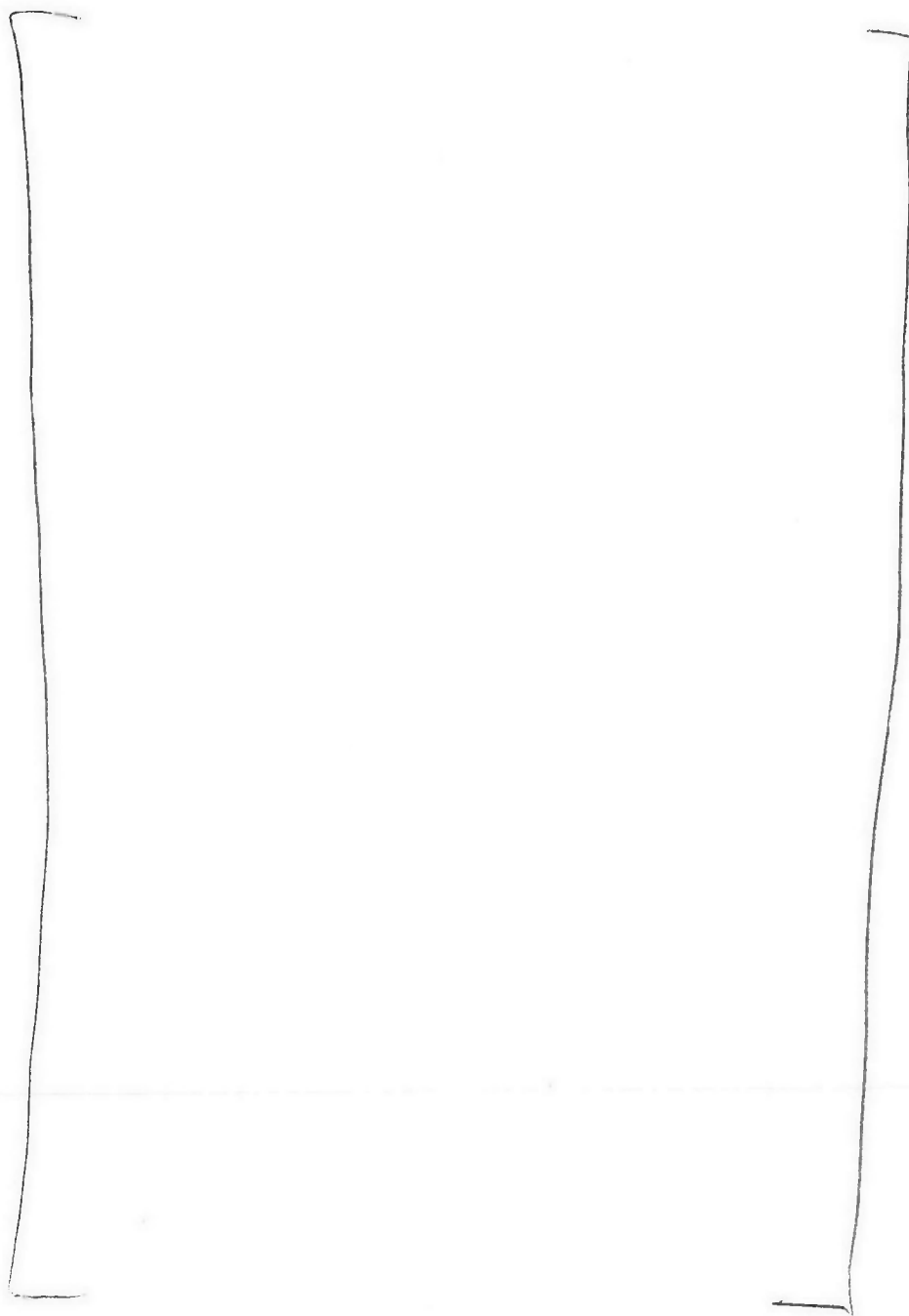
Figure 5.1 - Proton (^1H) NMR spectrum

Figure 5.2 - Proton (^1H) NMR spectrum (expanded)

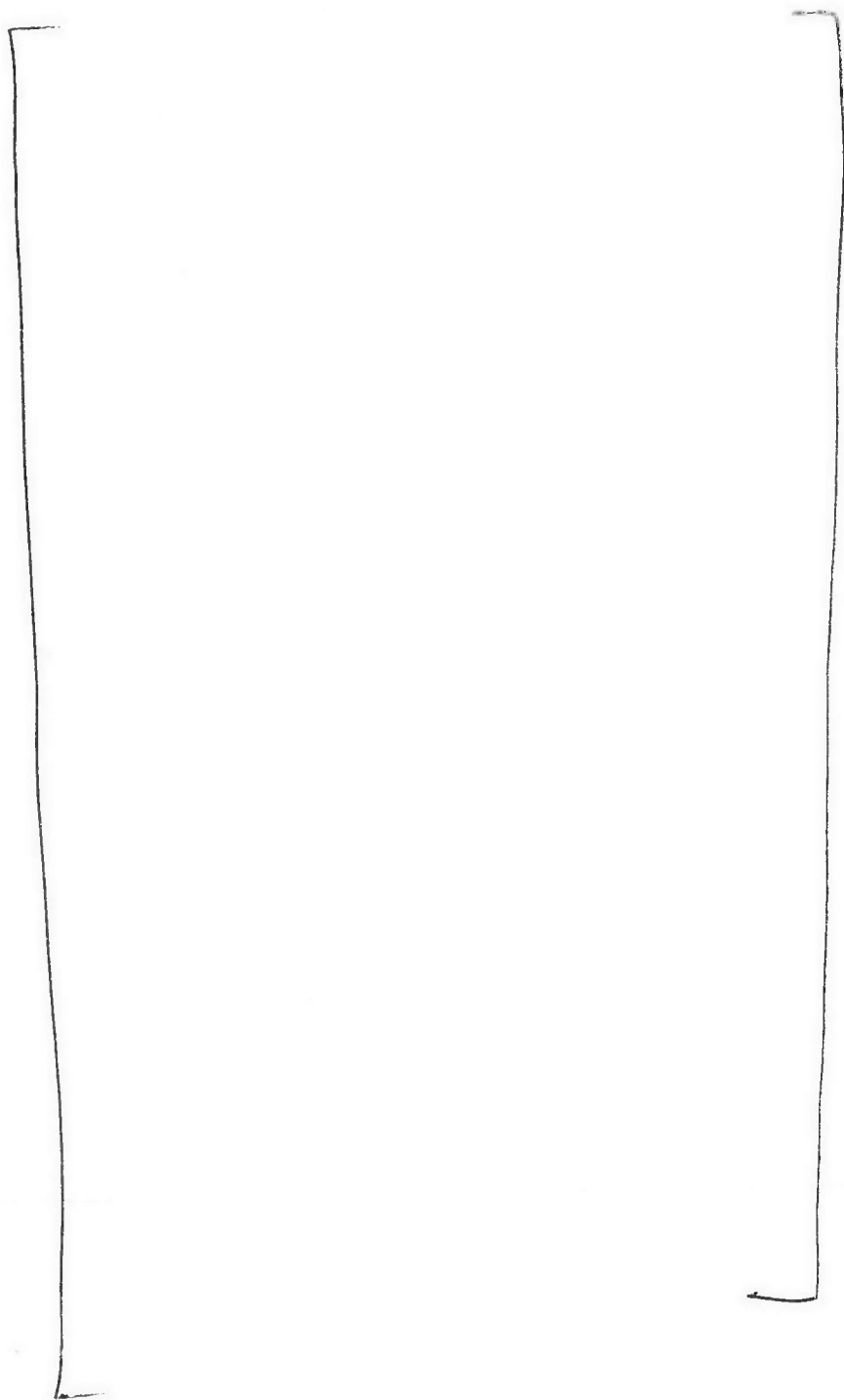
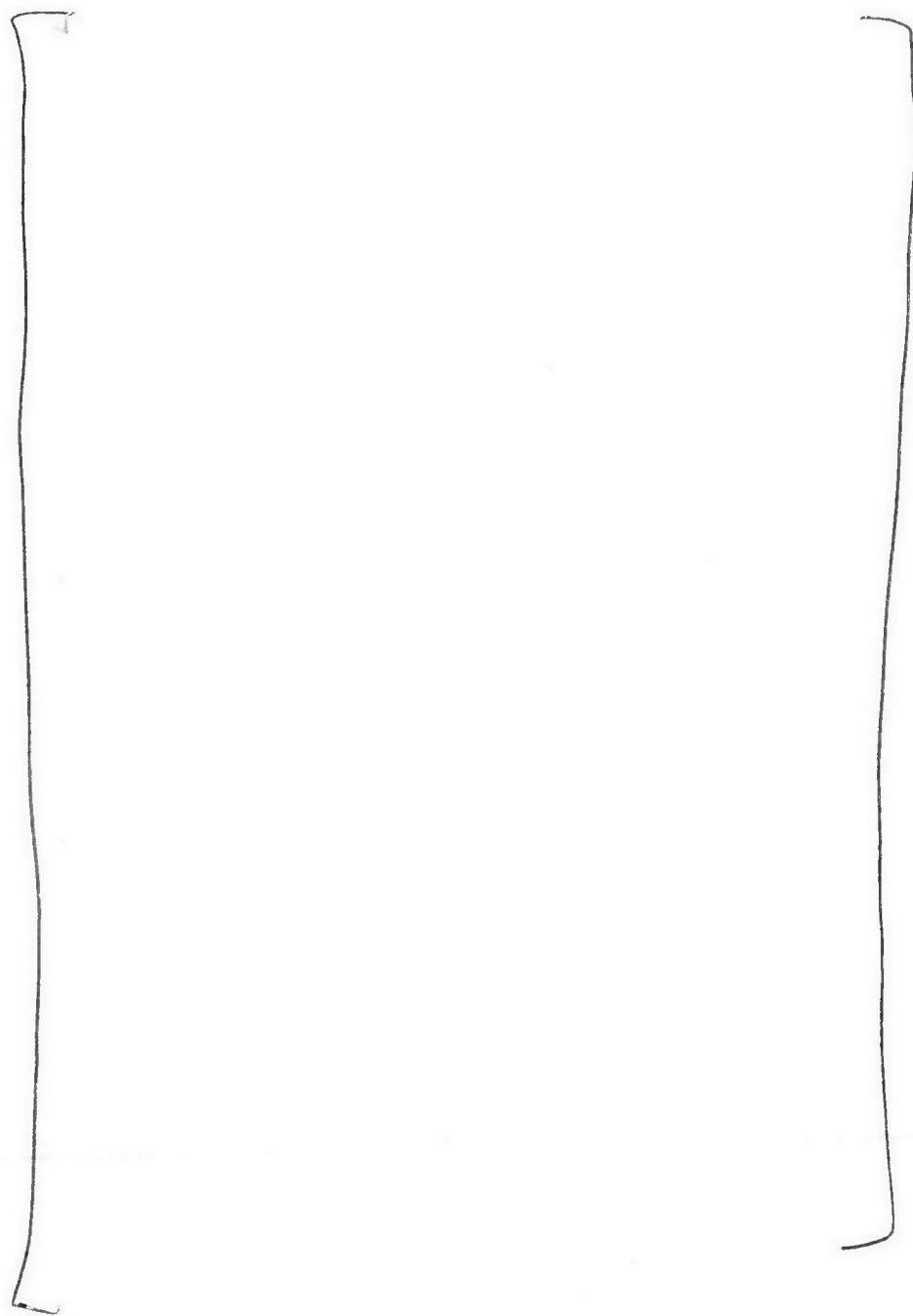


Figure 5.3 - Carbon (^{13}C) NMR spectrum

122

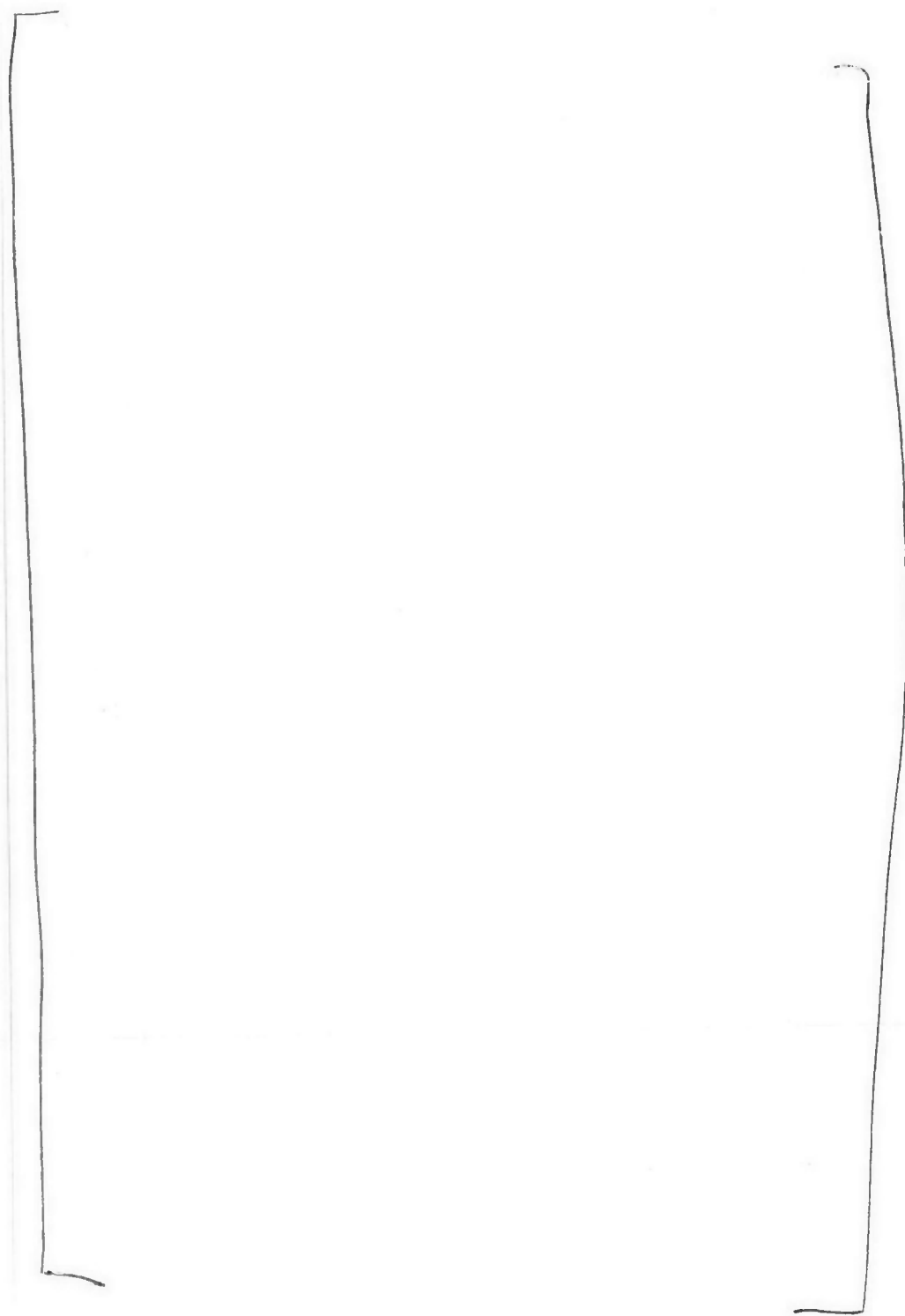
Figure 5.4 - Carbon (^{13}C) NMR spectrum (expanded)

Figure 5.5 - Carbon (^{13}C) NMR spectrum (expanded)

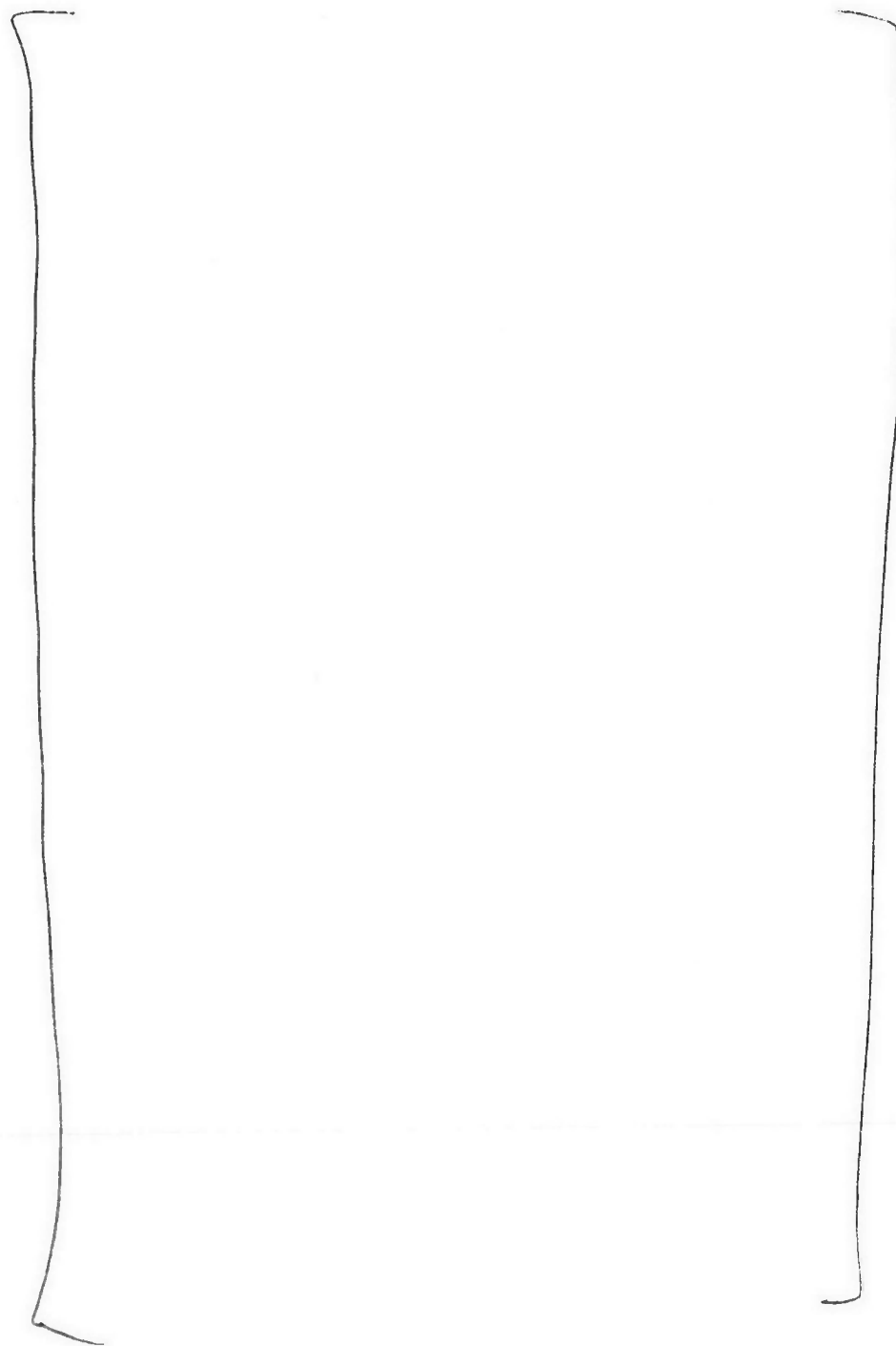
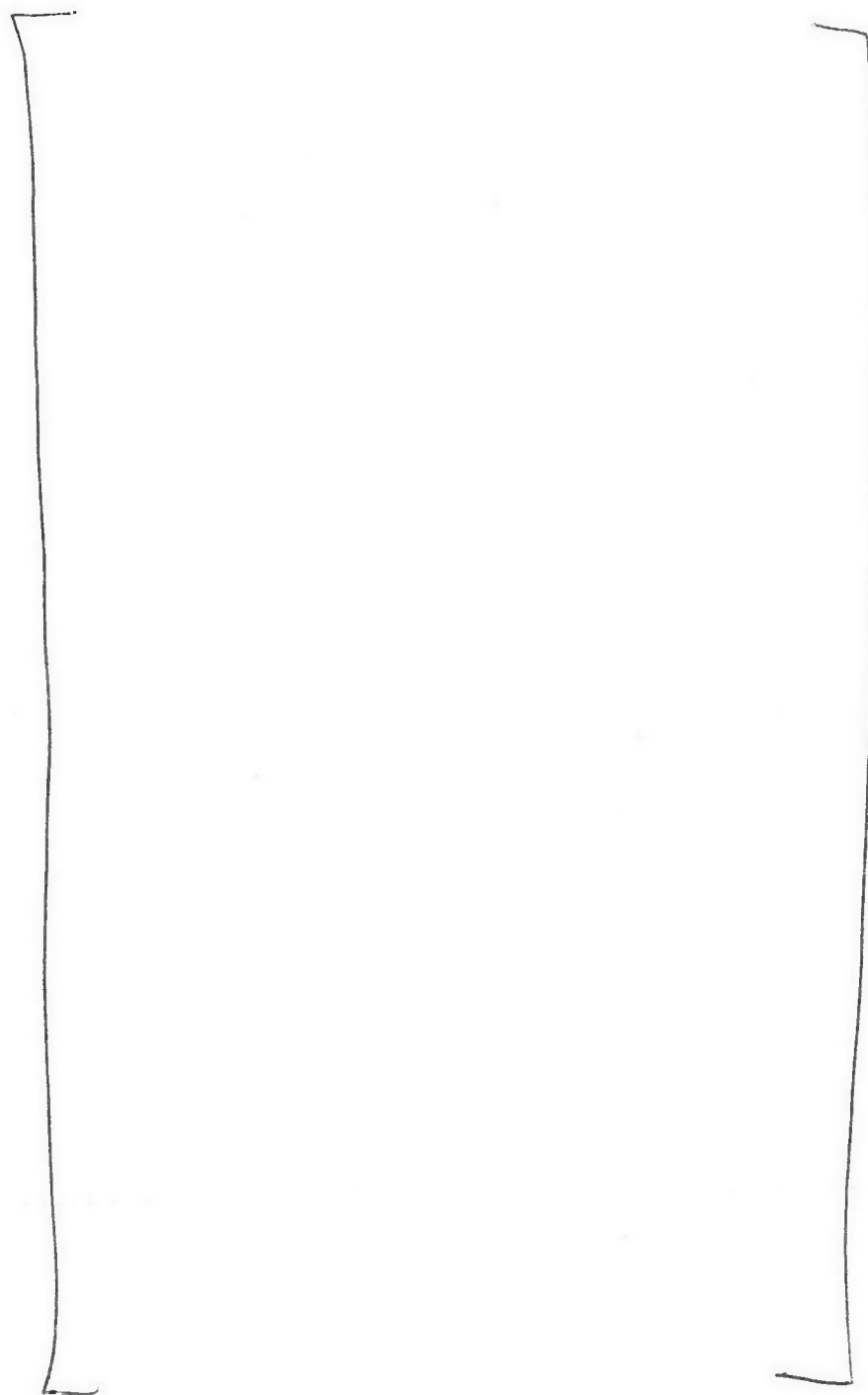


Figure 5.6 - Carbon (^{13}C) NMR DEPT 135 subspectrum



Peak assignments for the proton (^1H) NMR spectrum are shown in the following table:

Table 5.1

--

Peak assignments for the carbon (^{13}C) NMR spectrum are shown in the following table:

Table 5.2

--

5.4 Discussion

The poor solubility of the test material in typical NMR solvents necessitated the preparation of a saturated solution in deuterated THF. Therefore, peaks observed in the NMR spectra may be disproportionate to the composition due to the effect of concentrating more soluble components of the test material or minor impurities present in the test material.

The proton (^1H) and carbon (^{13}C) spectra were complex with multiple signals for methyl, methylene, methine and aromatic protons and carbons. This was considered to be due to the complex composition of the test material. Therefore it was not possible to positively identify the test material from the NMR spectra alone. However, no evidence was found in the NMR spectra that contradicted the proposed chemical composition of the test material.

5.5 Conclusion

The nuclear magnetic resonance spectra were consistent with the proposed chemical composition.

6. INFRARED SPECTRUM

6.1 Method

The infrared spectrum was acquired using a Perkin-Elmer Spectrum 1 FTIR spectrophotometer equipped with an attenuated total reflectance (ATR) accessory.

6.2 Procedure

The test material was scanned directly on the ATR accessory, over the range 4000 to 650 cm^{-1} .

6.3 Results

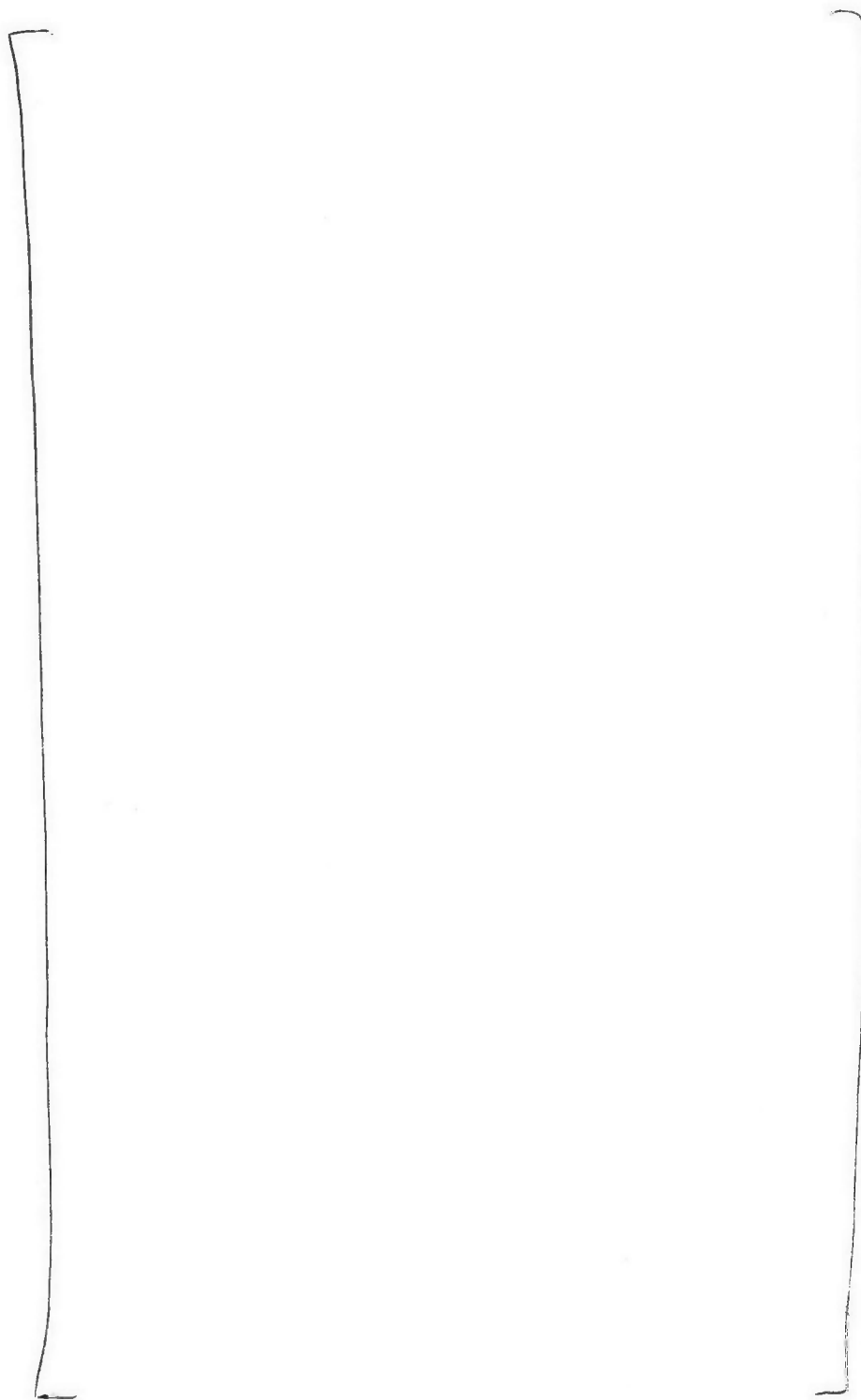
The major absorbances obtained from the infrared spectrum (Figure 6.1) are shown in the following table:

Table 6.1

Wavenumber (cm^{-1})	Assignment

6.4 Results

Figure 6.1 - Infrared Spectrum

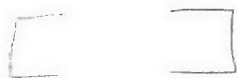


6.5 Discussion

It was not possible to positively identify the test material from the infrared spectrum alone. Many of the absorption bands present in the fingerprint region (900 to 1400 cm^{-1}) could not be assigned as these absorptions are uniquely characteristic to this test material. However, no evidence was found in the infrared spectrum that contradicted the proposed chemical composition of the test material.

6.6 Conclusion

The infrared spectrum of the test material was consistent with the proposed chemical composition.

REPORT**Acute Oral Toxicity Study in Rats**

Study Director: E. Rached

Test Facility: **Harlan Laboratories Ltd.**
Wölferstrasse 4
4414 Füllinsdorf / Switzerland

Sponsor: **Cheil Industrial Co., Ltd.**
(437-711) 332-2, Gocheon-dong
Uiwang-si
Gyeonggi-do / Korea

Study Identification: Harlan Laboratories Study C28591

Version: Final

Study Completion Date: 19-Feb-2009

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------------------------------	----

SIGNATURES

Study Director:

E. Rached

Eva Rached

Date: 19-FEB-2009

Management:

(for)

Dr. H. Fankhauser

Isaher

Date: 19-FEB-2009

GOOD LABORATORY PRACTICE**STATEMENT OF COMPLIANCE**

Harlan Laboratories Study: C28591
Test Item: ☐
Study Director: E. Rached
Study Title: Acute Oral Toxicity Study in Rats

The stability of the test item dilutions under the test conditions is unknown. The formulation trials were performed before the study initiation date. Therefore, they are excluded from this statement.

This study has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: E. Rached

Eva Rached
Date: 19 - FEB - 2009

QUALITY ASSURANCE GLP STATEMENT

Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen / Switzerland

Harlan Laboratories Study: C28591

Test Item:

[]

Study Director:

E. Rached

Study Title:

Acute Oral Toxicity Study in Rats

The general facilities and activities are inspected at least once a year and the results are reported to the responsible person and the management.

Study procedures, with exception of the formulation trials, were periodically inspected. The study plan and this report were audited by the Quality Assurance. The dates are given below.

Dates and Types of QA Inspections		Dates of Reports to the Study Director and Test Facility Management
02-Dec-2008	Study Plan	02-Dec-2008
18-Nov-2008	Process based (Test System, Test Item, Raw Data)	18-Nov-2008
17-Feb-2009	Report	17-Feb-2009

This statement also confirms that this final report reflects the raw data.

Quality Assurance:

S. van Dongen

S. van Dongen
Date: 19-Feb-2009

PREFACE

General Information

Test Item:

Study Title:

Sponsor:

Study Monitor:

Test Facility:

QA:

Acute Oral Toxicity Study in Rats

Cheil Industrial Co., Ltd.

(437-711) 332-2, Gocheon-dong

Uiwang-si

Gyeonggi-do / Korea

Ms. H. Lim

Chemtopia Co., Ltd.

No. 1407, Leaders Bldg.,

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Seocho-gu, Seoul 137-876 / South Korea

Harlan Laboratories Ltd.

Wölferstrasse 4

4414 Füllinsdorf / Switzerland

Harlan Laboratories Ltd.

Quality Assurance GLP

Zelgliweg 1

4452 Itingen / Switzerland

Responsibilities

Study Director:

Deputy Study Director:

Laboratory / Technical Coordinator:

E. Rached

G. Arcelin

C. Weng

Quality Assurance:

Head of QA:

T. Fink

Schedule

Experimental Starting Date:	03-Dec-2008
Termination (Necropsy):	24-Dec-2008 (females, 2000 mg/kg) 26-Dec-2008 (females, 2000 mg/kg)
Delivery of Animals:	03-Dec-2008 (females, 2000 mg/kg) 05-Dec-2008 (females, 2000 mg/kg)
Acclimatization:	03-Dec-2008 to 09-Dec-2008 (females, 2000 mg/kg) 05-Dec-2008 to 11-Dec-2008 (females, 2000 mg/kg)
Administration / Treatment:	10-Dec-2008 (females, 2000 mg/kg) 12-Dec-2008 (females, 2000 mg/kg)
Experimental Completion Date:	26-Dec-2008

Data Requirements / Test Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity – Acute Toxic Class Method", adopted 17th December 2001.
- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), B.1 tris. ACUTE ORAL TOXICITY – ACUTE TOXIC CLASS METHOD (Official Journal No L 142, 31/05/2008 p. 0158-0173).

Animal Welfare

This study was performed in an AAALAC-accredited laboratory in accordance with the Swiss Animal Protection Law under license no. 34.

Classification Guidelines

The test item was classified according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised Edition, 2007.



Archiving

Harlan Laboratories Ltd. (4452 Itingen / Switzerland) will retain the study plan, raw data, sample of test item(s) and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

1 SUMMARY

Two groups, each of three female HanRcc:WIST (SPF) rats, were treated with [] by oral gavage administration at a dosage of 2000 mg/kg body weight. The test item was formulated in PEG 300 at a concentration of 0.2 g/mL and administered at a dosing volume of 10 mL/kg.

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs within the first 30 minutes and approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15. Body weights were recorded on day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

All animals survived until the end of the study period.

No clinical signs were observed during the course of the study.

The body weight of the animals was within the range commonly recorded for this strain and age.

No macroscopic findings were recorded at necropsy.

The median lethal dose of [] after single oral administration to female rats, observed over a period of 14 days, is:

LD₅₀ (female rat): greater than 2000 mg/kg body weight

Based upon the results of this study and the classification criteria according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised Edition 2007, [] should not be classified with respect to acute oral toxicity.

2 PURPOSE

The purpose of this study was to assess the acute toxicity of [] when administered by a single oral gavage to rats, followed by an observation period of 14 days.

This study provides information for both hazard assessment and hazard classification purposes.

3 MATERIALS AND METHODS

3.1 Test System

Animals:	Rat, HanRcc: WIST(SPF)
Rationale:	Recognized by international guidelines as a recommended test system.
Breeder:	Harlan Laboratories Ltd. Laboratory Animal Services Wölferstrasse 4 4414 Füllinsdorf / Switzerland
Number of Animals per Group:	3 females
Total Number of Animals:	6 females
Age (when treated):	11 weeks
Body Weight Range (when treated):	185.1 g - 201.1 g
Identification:	Unique cage number and corresponding color-coded spots on the tail. The animals were marked at acclimatization start.
Randomization:	Selected by hand at time of delivery. No computer generated randomization program.
Acclimatization:	Under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

3.2 Allocation

The animals were distributed as follows:

Dose Levels	Group	Animal Numbers
2000 mg/kg	1	1-3
2000 mg/kg	2	4-6

3.3 Husbandry

Room Numbers:	0105 / Harlan Laboratories Ltd., Füllinsdorf
Conditions:	Standard Laboratory Conditions. Air-conditioned with 10-15 air changes per hour, and continuously monitored environment with ranges for room temperature 22 ± 3 °C and for relative humidity between 30-70 % (values above 70 % during cleaning process possible), automatically controlled light cycle of 12 hours light and 12 hours dark, music during the daytime light period.
Accommodation:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, 4132 Muttensz / Switzerland).
Diet:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 44/08 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) <i>ad libitum</i> (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose). Results of analyses for contaminants are archived at Harlan Laboratories Ltd.
Water:	Community tap water from Füllinsdorf <i>ad libitum</i> . Results of bacteriological, chemical and contaminant analyses are archived at Harlan Laboratories Ltd.

3.4 Test Item and Vehicle

3.4.1 Test Item

The following information was provided by the Sponsor:

Identification:	[]
Description:	Solid
Chemical Name:	[]
CAS No.:	[]
Batch Number:	20081010
Purity:	>99 %
Stability of Test Item:	Stable under storage conditions
Expiry Date:	26-Oct-2010
Stability of Test Item Dilution:	Unknown; is excluded from the statement of compliance.

Storage Conditions: At room temperature (range of 20 ± 5 °C, provided by Harlan Laboratories Ltd.), light protected.

Safety Precautions: Routine hygienic procedures were used to ensure the health and safety of the personnel.

3.4.2 Vehicle

The following information was provided by Harlan Laboratories Ltd.:

Identification: Polyethylene glycol 300 (PEG 300)

Description: Colorless viscous liquid

Lot number: 1349048

Source: FLUKA Chemie GmbH, 9471 Buchs / Switzerland

Stability of the Vehicle: Stable under storage conditions

Expiry Date: Apr-2010

Storage Conditions: At room temperature (range of 20 ± 5 °C), light protected.

Safety Precautions: Routine hygienic procedures were used to ensure the health and safety of the personnel.

PEG 300 was found to be a suitable vehicle.

The vehicle was chosen after a non-GLP solubility trial which was performed before the study initiation date. This formulation trial is excluded from the statement of compliance.

3.5 Preparation of Dose Formulations

Dose levels are in terms of the test item as supplied by the Sponsor.

The dose formulations were made shortly before each dosing occasion using a magnetic stirrer and a spatula as homogenizers.

The test item was weighed into a tared glass beaker on a suitable precision balance and the vehicle added (weight:volume).

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer.

3.6 Test Item Administration

The animals received a single dose of the test item by oral gavage administration at 2000 mg/kg body weight after being fasted for approximately 18 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing.

The dosing volume was 10 mL/kg body weight.

3.7 Rationale

Oral administration was considered to be an appropriate application method as it is a possible route of human exposure during manufacture, handling and use of the test item.

3.8 Observations

Viability / Mortality:

Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (in common with the clinical signs) and twice daily during days 2-15.

Clinical Signs:

Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1, depending on the occurrence of clinical signs of toxicity. Once daily during days 2-15.

Body Weights:

On test days 1 (prior to administration), 8 and 15.

3.9 Pathology

3.9.1 Necropsy

All animals were killed at the end of the observation period by carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. No organs or tissues were retained.

3.10 Statistical Analysis

No statistical analysis was used.

3.11 Data Compilation

Body weights were recorded on-line.

Mortality/viability and clinical signs were recorded on data sheets.

Macroscopic findings were compiled into the RCC Tox Computer System during recording.

The RCC Tox Computer System (RCC-Tox-Lims) had been validated with respect to data collection, storage and retrievability.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Viability / Mortality

(See Individual Tables on p. 20)

No deaths occurred during the study.

4.1.2 Clinical Signs

(See Individual Tables on p. 20)

No clinical signs were observed during the course of the study.

4.1.3 Body Weights

(See Individual Tables on p. 21)

The body weight of the animals was within the range commonly recorded for this strain and age.

4.2 Pathology

4.2.1 Macroscopic Findings

(See Individual Tables on p. 22)

No macroscopic findings were recorded at necropsy.

5 CONCLUSION

The median lethal dose of [] after single oral administration to female rats, observed over a period of 14 days, is:

LD₅₀ (female rat): greater than 2000 mg/kg body weight

Based upon the results of this study and the classification criteria according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised Edition 2007, [] should not be classified with respect to acute oral toxicity.



6 INDIVIDUAL TABLES

Mortality / Clinical Signs

Dose mg/kg bw	Animal No.	Sex	Signs	Test days																		
				1					2	3	4	5	6	7	8	9	10	11	12	13	14	15
				0.5*	1*	2*	3*	5*														
2000	1	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	2	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	3	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2000	4	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	5	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	6	F	No clinical signs	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Key: √ noted

* Examinations were performed within the first 30 minutes and 1, 2, 3 and 5 hours after treatment.

No clinical signs were evident in any animal during the acclimatization period.

BODY WEIGHTS (GRAM)

GROUP / SEX	ANIMAL	DAY 1	DAY 8	DAY 15
GROUP 1 / FEMALES (2000 MG/KG)	1	198.0	213.8	222.5
	2	188.5	206.5	217.1
	3	201.1	212.3	218.9
	MEAN	195.8	210.9	219.5
	ST.DEV.	6.6	3.8	2.8
	N	3	3	3
GROUP 2 / FEMALES (2000 MG/KG)	4	185.1	213.6	222.6
	5	195.7	223.6	233.1
	6	192.7	223.7	228.2
	MEAN	191.2	220.3	228.0
	ST.DEV.	5.5	5.8	5.2
	N	3	3	3

MACROSCOPICAL FINDINGS

FEMALES

GROUP 1 (2000 MG/KG)

ANIMAL 1

(SCHEDULED NECROPSY, 24-DEC-08, DAY 15 AFTER TREATMENT)

NO FINDINGS NOTED

ANIMAL 2

(SCHEDULED NECROPSY, 24-DEC-08, DAY 15 AFTER TREATMENT)

NO FINDINGS NOTED

ANIMAL 3

(SCHEDULED NECROPSY, 24-DEC-08, DAY 15 AFTER TREATMENT)

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS**FEMALES****GROUP 2 (2000 MG/KG)**

ANIMAL 4

(SCHEDULED NECROPSY, 26-DEC-08, DAY 15 AFTER TREATMENT)

NO FINDINGS NOTED

ANIMAL 5

(SCHEDULED NECROPSY, 26-DEC-08, DAY 15 AFTER TREATMENT)

NO FINDINGS NOTED

ANIMAL 6

(SCHEDULED NECROPSY, 26-DEC-08, DAY 15 AFTER TREATMENT)

NO FINDINGS NOTED

**ACUTE DERMAL IRRITATION
IN THE RABBIT**

PROJECT NUMBER: 2737/0007

AUTHOR: J Bradshaw

STUDY SPONSOR:

Cheil Industrial Inc
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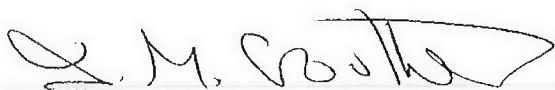
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

14 November 2008	Standard Test Method Compliance Audit
10 February 2009	Test Material Preparation
10 February 2009	Animal Preparation
10 February 2009	Dosing
10 February 2009	Assessment of Response
§ 30 April 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§ Evaluation specific to this study	



DATE: 19 JUN 2009

For the Quality Assurance Unit*

***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

.....
J Bradshaw BSc (Hons)
Study Director

DATE: 19 JUN 2009

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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ACUTE DERMAL IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the skin of the New Zealand White rabbit. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin Irritation) of Commission Regulation (EC) No. 440/2008

Results. 3-minute and 1-hour semi-occluded applications of the test material to the intact skin of one rabbit produced no evidence of skin irritation.

A single 4-hour, semi-occluded application of the test material to the intact skin of two rabbits produced no evidence of skin irritation.

Conclusion. The test material produced a primary irritation index of 0.0 and was classified as non-irritant to rabbit skin according to the Draize classification scheme.

ACUTE DERMAL IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following single, 3-minute, 1 and 4-hour, semi-occluded applications to the intact rabbit skin. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" (adopted 24 April 2002)
- Method B4 Acute Toxicity (Skin Irritation) of Commission Regulation (EC) No. 440/2008

The albino rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely skin irritancy potential of the test material to man.

The study was performed between 17 February 2009 and 27 February 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	[]
Description	:	white powder
Batch number	:	20090105
Date received	:	23 January 2009
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 1.

2.2 Preparation of Test Material

For the purpose of the study the test material was used as supplied.

The absorption of the test material was not determined.

2.3 Measurement of pH

The pH of the test material was determined prior to commencement of the study and found to be as follows:

Preparation	pH Measurement	
	immediately	after 10 minutes
10% w/w aqueous preparation of the test material	6.8	6.4

3. METHODS

3.1 Animals and Animal Husbandry

Two New Zealand White rabbits were supplied by Harlan Laboratories UK Limited, Bicester, Oxon, UK. At the start of the study the animals weighed 2.69 or 2.89 kg and were twelve to twenty weeks old. After an acclimatisation period of at least five days each animal was given a number unique within the study which was written with a black indelible marker-pen on the inner surface of the ear and on the cage label.

The animals were individually housed in suspended cages. Free access to mains drinking water and food (diet supplied by Harlan Teklad, Blackthorn, Bicester, Oxon, UK) was allowed throughout the study. The diet and drinking water were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

On the day before the test each rabbit was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study.

One rabbit was initially treated. Three suitable sites were selected on the back of the rabbit. At each test site a quantity of 0.5 g of the test material, moistened with 0.5 ml of distilled water, was introduced under a 2.5 cm x 2.5 cm cotton gauze patch and placed in position on the shorn skin. Each patch was secured in position with a strip of surgical adhesive tape. To prevent the animal interfering with the patches, the trunk of the rabbit was wrapped in an elasticated corset for the duration of the exposure period.

One patch was removed at each of three time points: 3 minutes, 1 hour and 4 hours after application. Any residual test material was removed by gentle swabbing with cotton wool soaked in distilled water.

After consideration of the skin reactions produced in the first animal, an additional animal was treated with 0.5 g of test material moistened with 0.5 ml of distilled water. One patch was applied to the back of the rabbit and was allowed to remain in contact with the skin for a period of four hours.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the following scale:

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	Value
No erythema.....	0
Very slight erythema (barely perceptible).....	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Oedema Formation	
No oedema.....	0
Very slight oedema (barely perceptible).....	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Any other skin reactions, if present, were also recorded.

Individual bodyweights were recorded on Day 0 (the day of dosing) and at the end of the observation period.

3.3 Interpretation of Results

Calculation of Primary Irritation Index and Grading of Irritancy Potential Using the Draize Scheme

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the two test rabbits (8 values) and this total was divided by four to give the primary irritation index of the test material. The test material was classified according to the following scheme devised by Draize J H (1959) "Dermal Toxicity" In: Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the United States, Austin, Texas, p.46-59:

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

If irreversible alteration of the dermal tissue is noted in any rabbit, as judged by the Study Director, which include ulceration and clear necrosis or signs of scar tissue, the test material is classified as corrosive to rabbit skin. Classification according to Draize may, therefore, not be applicable.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Skin Reactions

5.1.1 3-Minute Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 1.

No evidence of skin irritation was noted during the study.

5.1.2 1-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 1.

No evidence of skin irritation was noted during the study.

5.1.3 4-Hour Exposure Period

The individual scores for erythema/eschar and oedema are given in Table 2.

No evidence of skin irritation was noted during the study.

5.2 Bodyweight

Individual bodyweights and bodyweight changes are given in Table 3.

All animals showed expected gain in bodyweight during the study.

6. CONCLUSION

The test material produced a primary irritation index of 0.0 and was classified as NON-IRRITANT to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions Following 3-Minute and 1-Hour Exposures

Skin Reaction	Observation Time	Individual Scores - Rabbit Number and Sex	
		68248 Male	
		3-Minute Exposure	1-Hour Exposure
Erythema/Eschar Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0
Oedema Formation	1 Hour	0	0
	24 Hours	0	0
	48 Hours	0	0
	72 Hours	0	0

ACUTE DERMAL IRRITATION IN THE RABBIT

Table 2 Individual Skin Reactions Following 4-Hour Exposure

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex		Total
		68248 Male	68270 Male	
Erythema/Eschar Formation	1 Hour	0	0	(0)
	24 Hours	0	0	0
	48 Hours	0	0	(0)
	72 Hours	0	0	0
Oedema Formation	1 Hour	0	0	(0)
	24 Hours	0	0	0
	48 Hours	0	0	(0)
	72 Hours	0	0	0
Sum of 24 and 72-hour Readings (S) :		0		
Primary Irritation Index (S/4) :		0/4 = 0.0		
Classification :		NON-IRRITANT		

() = Total values not used for calculation of primary irritation index

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ACUTE DERMAL IRRITATION IN THE RABBIT

Table 3 Individual Bodyweights and Bodyweight Change

Rabbit Number and Sex	Individual Bodyweight (kg)		Bodyweight Change (kg)
	Day 0	Day 3	
68248 Male	2.89	2.90	0.01
68270 Male	2.69	2.88	0.19

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Appendix 1 Certificate of Analysis

Certificate of Analysis

1. TEST MATERIAL IDENTIFICATION

- 1) PRODUCT NAME :
- 2) TEST MATERIAL(LOT No.) : 20090105
- 3) QUANTITY : 100g
- 4) CHEMICAL NAME :
- 5) COMPOSITIONS OF CHEMICAL

- 6) PURITY : >99%
- 7) APPEARANCE : White Powder
- 8) MOLECULAR WEIGHT :
- 9) MOLECULAR FORMULA :

2. SOLUBILITY :

- 1) INSOLUBLE : H₂O
- 2) SOLUBLE : Toluene(Partially)

3. STORAGE :

- 1) Storage temperature : Room Temperature
- 2) Expiry date : Jan. 08, 2011

We hereby certify that the data stated here above are true and correct

Company(Manufacturer) : CHEIL INDUSTRIAL INC.

Address : (437-711) 332-2, Gocheon-dong, Uiwang-si, Gyeonggi-do, Korea

Date : Jan. 09, 2009

Name of person in charge

Sunghee Ahn



**Appendix 2 Statement of GLP Compliance in Accordance with Directive
2004/9/EC**



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority





ACUTE EYE IRRITATION IN THE RABBIT

PROJECT NUMBER: 2737/0008

AUTHOR: J Bradshaw

STUDY SPONSOR:

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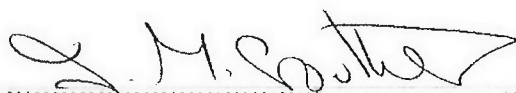
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

14 November 2008	Standard Test Method Compliance Audit
10 February 2009	Test Material Preparation
10 February 2009	Animal Preparation
10 February 2009	Dosing
10 February 2009	Assessment of Response
§ 01 May 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§ Evaluation specific to this study	



DATE: 19 JUN 2009

For the Quality Assurance Unit*

***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

.....
J Bradshaw BSc (Hons)
Study Director

DATE: 19 JUN 2009

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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ACUTE EYE IRRITATION IN THE RABBIT

SUMMARY

Introduction. The study was performed to assess the irritancy potential of the test material to the eye of the New Zealand White rabbit. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 405 "Acute Eye Irritation/Corrosion" (adopted 24 April 2002)
- Method B5 Acute Toxicity (Eye Irritation) of Commission Regulation (EC) No. 440/2008

Result. A single application of the test material to the non-irrigated eye of two rabbits produced moderate conjunctival irritation. One treated eye appeared normal at the 48-hour observation and the other treated eye appeared normal at the 72-hour observation.

Conclusion. The test material produced a maximum group mean score of 8.0 and was classified as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

ACUTE EYE IRRITATION IN THE RABBIT

1. INTRODUCTION

The study was performed to assess the irritancy potential of the test material following a single application to the rabbit eye. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 405 "Acute Eye Irritation/Corrosion" (adopted 24 April 2002)
- Method B5 Acute Toxicity (Eye Irritation) of Commission Regulation (EC) No. 440/2008

The albino rabbit has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely eye irritancy potential of the test material to man.

The study was performed between 04 March 2009 and 12 March 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	
Description	:	white powder
Batch number	:	20090105
Date received	:	23 January 2009
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 4.

2.2 Preparation of Test Material

For the purpose of the study the test material was used as supplied.

The absorption of the test material was not determined.

2.3 Measurement of pH

The pH of the test material was determined prior to commencement of the study and found to be as follows:

Preparation	pH Measurement	
	immediately	after 10 minutes
10% w/w aqueous preparation of the test material	6.8	6.4

3. METHODS

3.1 Animals and Animal Husbandry

Two New Zealand White rabbits were supplied by Harlan Laboratories UK Ltd, Bicester, Oxon, UK. At the start of the study the animals weighed 2.94 or 3.01 kg and were twelve to twenty weeks old. After an acclimatisation period of at least five days each animal was given a number unique within the study which was written with a black indelible marker-pen on the inner surface of the ear and on the cage label.

The animals were individually housed in suspended cages. Free access to mains drinking water and food (diet supplied by Harlan Teklad, Blackthorn, Bicester, Oxon, UK) was allowed throughout the study. The diet and drinking water were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

Immediately before the start of the test, both eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect with the aid of a light source from a standard ophthalmoscope. Only animals free of ocular damage were used.

Initially, a single rabbit was treated. A volume of 0.1 ml of the test material, which was found to weigh approximately 16 mg (as measured by gently compacting the required volume into an adapted syringe) was placed into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about one second immediately after treatment, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes. Immediately after administration of the test material, an assessment of the initial pain reaction was made according to the six point scale shown in Appendix 1.

After consideration of the ocular responses produced in the first treated animal, a second animal was treated.

Assessment of ocular damage/irritation was made approximately 1 hour and 24, 48 and 72 hours following treatment, according to the numerical evaluation given in Appendix 2, (from Draize J H (1977) "Dermal and Eye Toxicity Tests" In: Principles and Procedures for Evaluating the Toxicity of Household Substances, National Academy of Sciences, Washington DC p.48 to 49).

Any other ocular effects were also noted. Examination of the eye was facilitated by the use of the light source from a standard ophthalmoscope.

Individual bodyweights were recorded on Day 0 (the day of dosing) and at the end of the observation period.

3.3 Interpretation of Results

The numerical values corresponding to each animal, tissue and observation time were recorded. The data relating to the conjunctivae were designated by the letters A (redness), B (chemosis) and C (discharge), those relating to the iris designated by the letter D and those relating to the cornea by the letters E (degree of opacity) and F (area of cornea involved). For each tissue the score was calculated as follows:

$$\begin{aligned}\text{Score for conjunctivae} &= (A + B + C) \times 2 \\ \text{Score for iris} &= D \times 5 \\ \text{Score for cornea} &= (E \times F) \times 5\end{aligned}$$

Using the numerical data obtained a modified version of the system described by Kay J H and Calandra J C (1962), J. Soc. Cosmet. Chem. **13**, 281-289 (see Appendix 3) was used to classify the ocular irritancy potential of the test material. This was achieved by adding together the scores for the cornea, iris and conjunctivae for each time point for each rabbit. The group means of the total scores for each observation were calculated. The highest of these group means (the maximum group mean score) together with the persistence of the reactions enabled classification of the eye irritancy potential of the test material.

If evidence of irreversible ocular damage is noted, the test material will be classified as corrosive to the eye.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Ocular Reactions

Individual and group mean scores for ocular irritation are given in Table 1 and Table 2.

No corneal or effects were noted during the study.

Moderate conjunctival irritation was noted in both treated eyes one hour after treatment. Minimal conjunctival irritation was noted in both treated eyes at the 24-hour observation and in one treated eye at the 48-hour observation.

One treated eye appeared normal at the 48-hour observation and the other treated eye appeared normal at the 72-hour observation.

5.2 Bodyweight

Individual bodyweights and bodyweight changes are given in Table 3.

All animals showed expected gain in bodyweight during the study.

6. CONCLUSION

The test material produced a maximum group mean score of 8.0 and was classified as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to a modified Kay and Calandra classification system.

Table 1 Individual Scores and Individual Total Scores for Ocular Irritation

Rabbit Number and Sex	68290 Male					68298 Male				
	IPR = 2					IPR = 3				
Time After Treatment	1 Hour	24 Hours	48 Hours	72 Hours		1 Hour	24 Hours	48 Hours	72 Hours	
CORNEA										
E = Degree of Opacity	0	0	0	0		0	0	0	0	
F = Area of Cornea Involved	0	0	0	0		0	0	0	0	
Score (E x F) x 5	0	0	0	0		0	0	0	0	
IRIS										
D	0	0	0	0		0	0	0	0	
Score (D x 5)	0	0	0	0		0	0	0	0	
CONJUNCTIVAE										
A = Redness	2	1	1	0		2	1	0	0	
B = Chemosis	1	0	0	0		1	0	0	0	
C = Discharge	1	0	0	0		1	0	0	0	
Score (A + B + C) x 2	8	2	2	0		8	2	0	0	
Total Score	8	2	2	0		8	2	0	0	

IPR = Initial pain reaction

ACUTE EYE IRRITATION IN THE RABBIT

Table 2 Individual Total Scores and Group Mean Scores for Ocular Irritation

Rabbit Number and Sex	Individual Total Scores At:			
	1 Hour	24 Hours	48 Hours	72 Hours
68290 Male	8	2	2	0
68298 Male	8	2	0	0
Group Total	16	4	2	0
Group Mean Score	8.0	2.0	1.0	0.0

ACUTE EYE IRRITATION IN THE RABBIT

Table 3 Individual Bodyweights and Bodyweight Changes

Rabbit Number and Sex	Individual Bodyweight (kg)		Bodyweight Change (kg)
	Day 0	Day 3	
68290 Male	3.01	3.10	0.09
68298 Male	2.94	3.01	0.07

ACUTE EYE IRRITATION IN THE RABBIT**Appendix 1 Initial Pain Reaction**

When the material is instilled in the eye there may be an initial local pain reaction. The reaction will be graded as follows:

Class	Reaction by Animal	Descriptive Rating
0	No response	No initial pain
1	A few blinks only, normal within one or two minutes	Practically no initial pain
2	Rabbit blinks and tries to open eye, but reflex closes it	Slight initial pain
3	Rabbit holds eye shut and puts pressure on lids, may rub eye with paw	Moderate initial pain
4	Rabbit holds eye shut vigorously, may squeal	Severe initial pain
5	Rabbit holds eye shut vigorously, may squeal, claw at eye, jump and try to escape	Very severe initial pain

There is often no correlation between the initial pain and the subsequent eye irritation.

ACUTE EYE IRRITATION IN THE RABBIT

Appendix 2 Draize Scale for Scoring Ocular Irritation

1. CONJUNCTIVAE

(A)	Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
	Vessels normal	0
	Vessels definitely injected above normal	1
	More diffuse, deeper crimson red, individual vessels not easily discernible	2
	Diffuse beefy red	3
(B)	Chemosis	
	No swelling	0
	Any swelling above normal (includes nictitating membrane)	1
	Obvious swelling with partial eversion of lids	2
	Swelling with lids about half closed	3
	Swelling with lids half closed to completely closed	4
(C)	Discharge	
	No discharge	0
	Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
	Discharge with moistening of the lids and hairs just adjacent to lids	2
	Discharge with moistening of the lids and hairs a considerable area around the eye	3

THE TOTAL SCORE = (A + B + C) x 2

MAXIMUM TOTAL = 20

2. IRIS

(D)	Values	
	Normal	0
	Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
	No reaction to light, haemorrhage, gross destruction (any or all of these)	2

THE TOTAL SCORE = D x 5

MAXIMUM TOTAL = 10

3. CORNEA

(E)	Degree of Opacity (most dense area used)	
	No opacity	0
	Scattered or diffuse areas, details of iris clearly visible	1
	Easily discernible translucent areas, details of iris slightly obscured	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible	3
	Opaque, iris not discernible through the opacity	4
(F)	Area of Cornea Involved	
	One quarter (or less) but not zero	1
	Greater than one quarter but less than half	2
	Greater than half but less than three quarters	3
	Greater than three quarters, up to whole area	4

THE TOTAL SCORE = (E x F) x 5

MAXIMUM TOTAL = 80

MAXIMUM TOTAL SCORE POSSIBLE = 110

ACUTE EYE IRRITATION IN THE RABBIT

Appendix 3 Modified Kay and Calandra Interpretation of Eye Irritation Test

MAXIMUM MEAN SCORE		PERSISTENCE OF SCORE	DESCRIPTION RATING (AND CLASS)
0.0 to 0.5	Group mean total score at 24 hours = 0		Non-irritant (1)
	Group mean total score at 24 hours > 0		Practically non-irritant (2)
0.5 to 2.5	Group mean total score at 24 hours = 0		Practically non-irritant (2)
	Group mean total score at 24 hours > 0		Minimal irritant (3)
2.5 to 15	Group mean total score at 48 hours = 0		Minimal irritant (3)
	Group mean total score at 48 hours > 0		Mild irritant (4)
15 to 25	Group mean total score at 72 hours = 0		Mild irritant (4)
	Group mean total score at 72 hours > 0		Moderate irritant (5)
25 to 50		More than half of the individual total scores at 7 days 10 or less	Moderate irritant (5)
	Group mean total score at 7 days 20 or less	More than half of the individual total scores at 7 days > 10 but no individual total score at 7 days > 30	Moderate irritant (5)
		More than half of the individual total scores at 7 days > 10 and any individual score at 7 days > 30	Severe irritant (6)
	Group mean total score at 7 days > 20		Severe irritant (6)
50 to 80		More than half of the individual total scores at 7 days 30 or less	Severe irritant (6)
	Group mean total score at 7 days 40 or less	More than half of the individual total scores at 7 days > 30 but no individual total scores at 7 days > 60	Severe irritant (6)
		More than half of the individual total scores at 7 days > 30 and individual total score at 7 days > 60	Very severe irritant (7)
	Group mean total score at 7 days > 40		Very severe irritant (7)
80 to 100		More than half of the individual total scores at 7 days 60 or less	Very severe irritant (7)
	Group mean total score at 7 days 80 or less	More than half of the individual total scores at 7 days > 60 but no individual total score at 7 days > 100	Very severe irritant (7)
		More than half of the individual total scores at 7 days > 60 and individual total score at 7 days > 100	Extremely severe irritant (8)
	Group mean total score at 7 days > 80		Extremely severe irritant (8)
100 to 110	Group mean total score at 7 days 80 or less		Very severe irritant (7)
	Group mean total score at 7 days > 80		Extremely severe irritant (8)

Certificate of Analysis

1) PRODUCT NAME : []
2) TEST MATERIAL(LOT No.) : 20090105
3) QUANTITY : 100g
4) CHEMICAL NAME : []
5) COMPOSITIONS OF CHEMICAL

Chemical name	CAS No.	Contents

- We hereby certify that the data stated here above are true and correct

Sunghee Ahn

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**Appendix 5 Statement of GLP Compliance in Accordance with Directive
2004/9/EC**



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


4/3/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



LOCAL LYMPH NODE ASSAY IN THE MOUSE

PROJECT NUMBER: 2737/0009

AUTHOR: J Bradshaw

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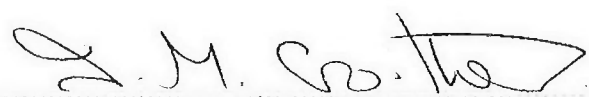
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

12 November 2008	Standard Test Method Compliance Audit
04 March 2009	Test Material Preparation
02 March 2009	Test System Preparation
05 March 2009	Animal Preparation
04 March 2009	Dosing
03 March 2009	Assessment of Response
§ 01 May 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§ Evaluation specific to this study	

 DATE: 19 JUN 2009
For the Quality Assurance Unit*

***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

J Bradshaw BSc (Hons)
Study Director

DATE: 19 JUN 2009

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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LOCAL LYMPH NODE ASSAY IN THE MOUSE

SUMMARY

Introduction. A study was performed to assess the skin sensitisation potential of the test material in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)
- Method B42 Skin Sensitisation (Local Lymph Node Assay) of Commission Regulation (EC) No. 440/2008

Methods. Following a preliminary screening test in which no clinical signs of toxicity were noted at a concentration of 25% w/w, this concentration was selected as the highest dose investigated in the main test of the Local Lymph Node Assay. Three groups, each of four animals, were treated with 50 µl (25 µl per ear) of the test material as a suspension in propylene glycol at concentrations of 25%, 10% or 5% w/w. A further group of four animals was treated with propylene glycol alone.

Results. The Stimulation Index expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

Concentration (% w/w) in propylene glycol	Stimulation Index	Result
5	1.18	Negative
10	1.16	Negative
25	1.45	Negative

Conclusion. The test material was considered to be a non-sensitiser under the conditions of the test.

LOCAL LYMPH NODE ASSAY IN THE MOUSE

1. INTRODUCTION

A study was performed to assess the skin sensitisation potential of the test material in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)
- Method B42 Skin Sensitisation (Local Lymph Node Assay) of Commission Regulation (EC) No. 440/2008

The assay has undergone extensive inter-laboratory validation and has been shown to reliably detect test materials that are moderate to strong sensitisers.

The strain of mouse used in these laboratories has been shown to produce satisfactory responses using known sensitisers and non-sensitisers during the in-house validation. The results of routine positive control studies are shown in Appendix 1 and Appendix 2. The results of the study are believed to be of value in predicting the sensitisation potential of the test material to man.

The study was performed between 02 March 2009 and 24 March 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	[]
Description	:	white powder
Batch number	:	20090105
Date received	:	23 January 2009
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 4.

2.2 Preparation of Test Material

For the purpose of the study, the test material was freshly prepared as a suspension in propylene glycol. This vehicle was chosen as it produced the highest concentration that was suitable for dosing. The concentrations used are given in the procedure section. The vehicle determination record is included as Appendix 3.

Determination, by analysis, of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Female CBA/Ca (CBA/CaOlaHsd) strain mice were supplied by Harlan Laboratories UK Limited, Bicester, Oxon, UK. On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals were in the weight range of 15 to 23 g, and were eight to twelve weeks old.

The animals were individually housed in suspended solid-floor polypropylene cages furnished with softwood woodflakes. Free access to mains tap water and food (2014 Teklad Global Rodent diet supplied by Harlan Teklad, Blackthorn, Bicester, Oxon, UK) was allowed throughout the study.

The temperature and relative humidity were controlled to remain within target ranges of 19 to 25°C and 30 to 70%, respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of

air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06.00 to 18.00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

3.2.1 Preliminary Screening Test

Using available information regarding the irritancy potential of the test material, a preliminary screening test was performed using one mouse. The mouse was treated by daily application of 25 µl of the test material at a concentration of 25% w/w in propylene glycol, to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The mouse was observed twice daily on Days 1, 2 and 3 and once daily on Days 4, 5 and 6. Any signs of toxicity or excessive local irritation noted during this period were recorded. The bodyweight was recorded on Day 1 (prior to dosing) and on Day 6.

3.2.2 Main Test

3.2.2.1 Test Material Administration

Groups of four mice were treated with the test material at concentrations of 25%, 10% or 5% w/w in propylene glycol. The preliminary screening test suggested that the test material would not produce systemic toxicity or excessive local irritation at the highest suitable concentration. The mice were treated by daily application of 25 µl of the appropriate concentration of the test material to the dorsal surface of each ear for three consecutive days (Days 1, 2, 3). The test material formulation was administered using an automatic micropipette and spread over the dorsal surface of the ear using the tip of the pipette.

A further group of four mice received the vehicle alone in the same manner.

3.2.2.2 ³H-Methyl Thymidine Administration

Five days following the first topical application of the test material (Day 6) all mice were injected via the tail vein with 250 µl of phosphate buffered saline (PBS) containing ³H-methyl thymidine (³HTdR: 80 µCi/ml, specific activity 2.0 Ci/mmol, GE Healthcare UK Ltd) giving a total of 20 µCi to each mouse.

3.2.2.3 Observations

Clinical Observations: All animals were observed twice daily on Days 1, 2 and 3 and on a daily basis on Days 4, 5 and 6. Any signs of toxicity or signs of ill health during the test were recorded.

Bodyweights: The bodyweight of each mouse was recorded on Day 1 (prior to dosing) and Day 6 (prior to termination).

3.2.2.4 Terminal Procedures

Termination: Five hours following the administration of ³HTdR all mice were killed by carbon dioxide asphyxiation. The draining auricular lymph nodes from the four mice were excised and pooled for each experimental group. For each group 1 ml of PBS was added to the pooled lymph nodes.

Preparation of Single Cell Suspension: A single cell suspension of pooled lymph node cells was prepared by gentle mechanical disaggregation through a 200-mesh stainless steel gauze. The lymph node cells were rinsed through the gauze with 4 ml of PBS into a petri dish labelled with the project number and dose concentration. The lymph node cell suspension was transferred to a centrifuge tube. The petri dish was washed with an additional 5 ml of PBS to remove all remaining lymph node cells and these were added to the centrifuge tube. The pooled lymph node cells were pelleted at 1400 rpm (approximately 190 g) for ten minutes. The pellet was resuspended in 10 ml of PBS and re-pelleted. To precipitate out the radioactive material, the pellet was resuspended in 3 ml of 5% Trichloroacetic acid (TCA).

Determination of $^3\text{HTdR}$ Incorporation: After approximately eighteen hours incubation at approximately 4°C , the precipitates were recovered by centrifugation at 2100 rpm (approximately 450 g) for ten minutes, resuspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid (Optiphase 'Trisafe'). $^3\text{HTdR}$ incorporation was measured by β -scintillation counting. The "Poly QTM" vials containing the samples and scintillation fluid were placed in the sample changer of the scintillator and left for approximately twenty minutes. The purpose of this period of time in darkness was to reduce the risk of luminescence, which has been shown to affect the reliability of the results. After approximately twenty minutes, the vials were shaken vigorously. The number of radioactive disintegrations per minute was then measured using the Beckman LS6500 scintillation system (Beckman Instruments Inc, Fullerton, CA, USA).

3.3 Interpretation of Results

The proliferation response of lymph node cells was expressed as the number of radioactive disintegrations per minute per lymph node (disintegrations per minute/node) and as the ratio of $^3\text{HTdR}$ incorporation into lymph node cells of test nodes relative to that recorded for the control nodes (Stimulation Index).

The test material will be regarded as a sensitiser if at least one concentration of the test material results in a threefold or greater increase in $^3\text{HTdR}$ incorporation compared to control values. Any test material failing to produce a threefold or greater increase in $^3\text{HTdR}$ incorporation will be classified as a "non-sensitiser".

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Preliminary Screening Test

Clinical observations, bodyweight and mortality data are given in Table 1.

No signs of systemic toxicity were noted.

Based on this information the dose levels selected for the main test were 25%, 10% and 5% w/w in propylene glycol.

5.2 Main Test

5.2.1 Estimation of the Proliferative Response of Lymph Node Cells

The radioactive disintegrations per minute per lymph node and the stimulation index are given in Table 2.

A stimulation index of less than 3 was recorded for the three concentrations of the test material (25%, 10% and 5% w/w in propylene glycol).

5.2.2 Clinical Observations and Mortality Data

Individual clinical observations and mortality data for test and control animals are given in Table 3.

There were no deaths. No signs of systemic toxicity were noted in the test or control animals during the test.

5.2.3 Bodyweight

Individual bodyweights and bodyweight changes for test and control animals are given in Table 4.

Bodyweight changes of the test animals between Day 1 and Day 6 were comparable to those observed in the corresponding control group animals over the same period.

6. CONCLUSION

The test material was considered to be a non-sensitiser under the conditions of the test.

LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 1 **Clinical Observations, Bodyweight and Mortality Data – Preliminary Screening Test**

Concentration (% w/w) in propylene glycol	Animal Number	Bodyweight (g)		Day								
				1		2		3		4	5	6
		Day 1	Day 6	Pre- Dose	Post Dose	Pre- Dose	Post Dose	Pre- Dose	Post Dose			
25	S-1	16	17	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

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LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 2 Disintegrations per Minute, Disintegrations per Minute/Node and Stimulation Index

Concentration (% w/w) in propylene glycol	dpm	dpm/Node ^a	Stimulation Index ^b	Result
Vehicle	3363.26	420.41	na	na
5	3976.04	497.01	1.18	Negative
10	3916.15	489.52	1.16	Negative
25	4892.47	611.56	1.45	Negative

dpm = Disintegrations per minute

a = Disintegrations per minute/node obtained by dividing the disintegrations per minute value by 8
(total number of lymph nodes)

b = Stimulation Index of 3.0 or greater indicates a positive result

na = Not applicable

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LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 3 Individual Clinical Observations and Mortality Data

Concentration (% w/w) in propylene glycol	Animal Number	Day 1		Day 2		Day 3		Day 4	Day 5	Day 6
		Pre- Dose	Post Dose	Pre- Dose	Post Dose	Pre- Dose	Post Dose			
Vehicle	1-1	0	0	0	0	0	0	0	0	0
	1-2	0	0	0	0	0	0	0	0	0
	1-3	0	0	0	0	0	0	0	0	0
	1-4	0	0	0	0	0	0	0	0	0
5	2-1	0	0	0	0	0	0	0	0	0
	2-2	0	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0	0
	2-4	0	0	0	0	0	0	0	0	0
10	3-1	0	0	0	0	0	0	0	0	0
	3-2	0	0	0	0	0	0	0	0	0
	3-3	0	0	0	0	0	0	0	0	0
	3-4	0	0	0	0	0	0	0	0	0
25	4-1	0	0	0	0	0	0	0	0	0
	4-2	0	0	0	0	0	0	0	0	0
	4-3	0	0	0	0	0	0	0	0	0
	4-4	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

LOCAL LYMPH NODE ASSAY IN THE MOUSE

Table 4 Individual Bodyweights and Bodyweight Changes

Concentration (% w/w) in propylene glycol	Animal Number	Bodyweight (g)		Bodyweight Change (g)
		Day 1	Day 6	
Vehicle	1-1	17	16	-1
	1-2	16	18	2
	1-3	17	18	1
	1-4	16	17	1
5	2-1	18	18	0
	2-2	15	15	0
	2-3	16	17	1
	2-4	19	19	0
10	3-1	17	18	1
	3-2	18	19	1
	3-3	17	18	1
	3-4	16	17	1
25	4-1	19	18	-1
	4-2	18	19	1
	4-3	17	16	-1
	4-4	16	17	1

LOCAL LYMPH NODE ASSAY IN THE MOUSE

Appendix 1 Current Positive Control Study for the Local Lymph Node Assay

Introduction. A study was performed to assess the sensitivity of the strain of mouse used at these laboratories to a known sensitiser. The method was designed to meet the requirements of the following:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitisation: Local Lymph Node Assay" (adopted 24 April 2002)
- Method B42 Skin Sensitisation (Local Lymph Node Assay) of Commission Regulation (EC) No. 440/2008

Test Material: Phenylacetaldehyde (90%)
Project number: 0039/1079
Study dates: 20 March 2009 to 26 March 2009

Methods. One group of five animals was treated with 50 µl (25 µl per ear) of Phenylacetaldehyde (90%) as a solution in propylene glycol at a concentration of 2.5% v/v. A further control group of five animals was treated with propylene glycol alone.

Results. The Stimulation Index expressed as the mean radioactive incorporation for the treatment group divided by the mean radioactive incorporation of the vehicle control group is as follows:

Concentration (% v/v) in propylene glycol	Stimulation Index	Result
2.5	8.00	Positive

Conclusion. Phenylacetaldehyde (90%) was considered to be a sensitiser under the conditions of the test.

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LOCAL LYMPH NODE ASSAY IN THE MOUSE

Appendix 2 Summary of Positive Control Data for the Local Lymph Node Assay•

Project Number	Start Date	Finish Date	Test Material	Concentration	Vehicle	Stimulation Index ^a	Classification ^b
0039/1037	23/05/08	29/05/08	α -Hexylcinnamaldehyde	5%, 10%, 25% v/v	liquid paraffin BP	1.73, 2.32, 4.58	Positive
0039/1038	05/06/08	11/06/08	α -Hexylcinnamaldehyde	5%, 10%, 25% v/v	polyethylene glycol 300	2.07, 4.22, 10.92	Positive
0039/1057	17/10/08	23/10/08	α -Hexylcinnamaldehyde	15% v/v	acetone/olive oil 4:1	6.55	Positive
0039/1058	29/10/08	04/11/08	α -Hexylcinnamaldehyde	15% v/v	dimethyl formamide	4.55	Positive
0039/1059	29/10/08	04/11/08	α -Hexylcinnamaldehyde	15% v/v	butanone	4.05	Positive
0039/1060	05/11/08	17/12/08	α -Hexylcinnamaldehyde	15% v/v	dimethyl sulphoxide	5.73	Positive
0039/1061	05/11/08	11/11/08	α -Hexylcinnamaldehyde	15% v/v	acetone	4.21	Positive
0039/1062	05/11/08	11/11/08	α -Hexylcinnamaldehyde	15% v/v	ethanol/distilled water 7:3	9.49	Positive
0039/1078	18/03/09	24/03/09	2,4-Dinitrobenzenesulfonic acid, sodium salt	10% w/w	1% pluronic L92 in distilled water	13.71	Positive
0039/1079	20/03/09	26/03/09	Phenylacetaldhyde (90%)	2.5% v/v	propylene glycol	8.00	Positive
0039/1082	17/04/09	23/04/09	α -Hexylcinnamaldehyde	50% v/v	cotton seed oil	6.54	Positive
0039/1083	16/04/09	22/04/09	α -Hexylcinnamaldehyde	15% v/v	butanone	4.08	Positive
0039/1084	17/04/09	23/04/09	α -Hexylcinnamaldehyde	15% v/v	dimethyl sulphoxide	4.60	Positive

- = Standard Test Method 599 ('individual' nodes)
- a = Ratio of test to control lymphocyte proliferation
- b = Stimulation index greater than 3.0 indicates a positive result

PROJECT NUMBER: 2737/0009

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Appendix 3 Vehicle Determination Record

Test Material Concentration	Vehicle*							
	acetone/ olive oil (4:1)	dimethyl formamide	butanone	dimethyl sulphoxide	acetone	ethanol/ distilled water (7:3)	1% pluronic L92 in distilled water	propylene glycol
50% 0.5 g (test material) + 0.5 g (vehicle)	X 1	X 1	X 1	X 1	X 1	X 1	X 1	X
25% 1 g of 50% dilution made up to 2 g	X 1	X 1	X 1	X 1	X 1	X 1	X 1	✓ suspension 1,2

* = Suitable for dosing if formulation is a solution or fine homogenous suspension which can be administered via a micropipette
 X = Not suitable for dosing
 ✓ = Suitable for dosing
 1 = Vortex mixer
 2 = Ultra turrax

Appendix 4 Certificate of Analysis

Certificate of Analysis

1. TEST MATERIAL IDENTIFICATION

- 1) PRODUCT NAME : []
2) TEST MATERIAL(LOT No.) : 20090105
3) QUANTITY : 100g
4) CHEMICAL NAME : []
5) COMPOSITIONS OF CHEMICAL

Chemical name	CAS No.	Contents
---------------	---------	----------

- 6) PURITY : >99%
7) APPEARANCE : White Powder
8) MOLECULAR WEIGHT []
9) MOLECULAR FORMULA []

2. SOLUBILITY :

- 1) INSOLUBLE : H₂O
2) SOLUBLE : Toluene(Partially)

3. STORAGE :

- 1) Storage temperature : Room Temperature
2) Expiry date : Jan. 08, 2011

We hereby certify that the data stated here above are true and correct

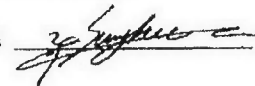
Company(Manufacturer) : CHEIL INDUSTRIAL INC.

Address : (437-711) 332-2, Gocheon-dong, Uiwang-si, Gyeonggi-do, Korea

Date : Jan. 09, 2009

Name of person in charge

Sunghee Ahn



**Appendix 5 Statement of GLP Compliance in Accordance with Directive
2004/9/EC**



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM
GOOD LABORATORY PRACTICE**

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

STUDY NUMBER 1228602

SALMONELLA TYPHIMURIUM
AND
ESCHERICHIA COLI
REVERSE MUTATION ASSAY

WITH



REPORT

STUDY COMPLETION DATE:
APRIL 01, 2009

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1 COPY OF GLP CERTIFICATE

HESSEN



Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility

☐ Prüfstandort/Test site

RCC – Cytotest Cell Research GmbH

RCC – Cytotest Cell Research GmbH

In den Leppsteinswiesen 19

64380 Rossdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise

(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

- 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften
- 3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
- 6 Prüfungen zur Bestimmung von Rückständen
- 8 Analytische Prüfungen an biologischen Materialien
- 9 Virussicherheitsprüfungen

- 2 Toxicity studies
- 3 Mutagenicity studies
- 6 Residues
- 8 Analytical studies on biological materials
- 9 Virus validation studies

02.09.2006

Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Th. Zimmermann, Referent, Wiesbaden, den 19. Januar 2007
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)



Hess. Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden

(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

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3 PREFACE

3.1 General

Title: Salmonella typhimurium and Escherichia coli
Reverse Mutation Assay with []

Sponsor: Cheil Industrial Co., Ltd.
Sunghee Ahn
(437-711) 332-2, Gocheon-dong
Uiwang-si, Gyeonggi-do, Korea

Study Monitor: Ms. Jin-Sil Bak
Chemtopia Co. Ltd.
No. 1407, Leaders Building
1599-11, Seocho3-dong
Seocho-gu
Seoul 137-876, Korea

Test Facility: Harlan
Cytotest Cell Research GmbH (Harlan CCR)
(former RCC Cytotest Cell Research GmbH)
In den Leppsteinswiesen 19
64380 Rossdorf/Germany

Contracting Institute: Harlan Laboratories Ltd.
4452 Itingen
Switzerland

Reference Number: C28613

3.2 Responsibilities

Study Director: Dipl. Biol. Andrea Sokolowski

Deputy Study Director: Dr. Hans-Eric Wollny

Management: Dr. Wolfgang Völkner

Head of Quality Assurance Unit: Frauke Hermann

3.3 Schedule

Experimental Starting Date: February 09, 2009

Experimental Completion Date: February 23, 2009

3.4 Project Staff Signatures

Study Director

Dipl. Biol. Andrea Sokolowski



Date: April 01, 2009

Management

Dr. Wolfgang Völkner



Date: April 01, 2009

3.5 Good Laboratory Practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

3.6 Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997

"Commission Regulation (EC) No. 440/2008 B13/14", dated May 30, 2008

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3.7 Archiving

Harlan CCR will archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

No data will be discarded without the sponsor's consent.

3.8 Deviations to Study Plan

3.5 Guidelines (page 5)

Previous:

"Council Regulation (EC) No. 440/2008 B13/14", dated May 30, 2008

New:

"Commission Regulation (EC) No. 440/2008 B13/14", dated May 30, 2008

Reason for the Alteration:

Updating

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4 STATEMENT OF COMPLIANCE

Study Number: 1228602

Test Item:



Study Director:

Dipl. Biol. Andrea Sokolowski

Title:

Salmonella typhimurium and Escherichia coli
Reverse Mutation Assay with SH-1

This study performed in the test facility of Harlan CCR was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

Harlan C C R

Dipl. Biol. Andrea Sokolowski

Date: April 01, 2009

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1228602

Test Item:



Study Director:

Dipl. Biol. Andrea Sokolowski

Title:

Salmonella typhimurium and Escherichia coli
Reverse Mutation Assay with []

The general facilities and activities of Harlan CCR are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	December 18, 2008	December 18, 2008
1 st Amendment to Study Plan:	April 01, 2009	April 01, 2009
<u>Process Inspection</u>		
Application:	February 11, 2009	February 11, 2009
Report:	April 01, 2009	April 01, 2009

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit

Frauke Hermann

Date: April 01, 2009

6 SUMMARY OF RESULTS

This study was performed to investigate the potential of [] to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with [] at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

6.1 Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, [] is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

7 OBJECTIVE

7.1 Aims of the Study

The experiments were performed to assess the potential of the test item to induce gene mutations by means of two independent *Salmonella typhimurium* and *Escherichia coli* reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

7.2 Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria (3). They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The *Salmonella typhimurium* histidine (his) and the *E. coli* tryptophan (trp) reversion system measures $\text{his}^- \rightarrow \text{his}^+$ and $\text{trp}^- \rightarrow \text{trp}^+$ reversions, respectively. The *S. typhimurium* and *Escherichia coli* strains are constructed to differentiate between base pair (TA 1535, TA 100, and WP2 uvrA) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect at least 6 dose levels with adequately spaced intervals were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens were tested in parallel to the test item.

8 MATERIALS AND METHODS

8.1 Test Item

Internal Test Item Number: S 9613 11

The test item and the information concerning the test item were provided by the sponsor.

Identity:

Chemical Name:

Batch No.: 20081010

Purity: > 99 %

Stability in Solvent: Not indicated by the sponsor

Storage: At room temperature

Expiration Date: January 08, 2011

On the day of the experiment, the test item [] was suspended in DMSO (MERCK, D-64293 Darmstadt; purity > 99 %). The solvent was chosen because of its solubility properties and its relative non-toxicity to the bacteria (5).

Precipitation of the test item was observed in the test tubes from 1000 - 5000 µg/plate in experiment I, and at 2500 and 5000 µg/plate in experiment II. Precipitation of the test item was also observed on the incubated agar plates at 2500 and 5000 µg/plate in both experiments. The undissolved particles had no influence on the data recording.

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8.2 Controls

8.2.1 Negative Controls

Concurrent untreated and solvent controls were performed.

8.2.2 Positive Control Substances

Without metabolic activation

Strains: TA 1535, TA 100
Name: sodium azide, NaN_3
Supplier: SERVA, D-69042 Heidelberg
Catalogue No.: 30175
Purity: at least 99 %
Dissolved in: deionised water
Concentration: 10 $\mu\text{g}/\text{plate}$

Strains: TA 1537, TA 98
Name: 4-nitro-o-phenylene-diamine, 4-NOPD
Supplier: SIGMA, D-82041 Deisenhofen
Catalogue No.: N 9504
Purity: > 99.9 %
Dissolved in: DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration: 10 $\mu\text{g}/\text{plate}$ in TA 98, 50 $\mu\text{g}/\text{plate}$ in TA 1537

Strain: WP2 uvrA
Name: methyl methane sulfonate, MMS
Supplier: Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue No.: 820775
Purity: > 99.0 %
Dissolved in: deionised water
Concentration: 3 $\mu\text{L}/\text{plate}$

With metabolic activation

Strains: TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA
Name: 2-aminoanthracene, 2-AA
Supplier: SIGMA, D-82041 Deisenhofen
Catalogue No.: A 1381
Purity: 97.5 %
Dissolved in: DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration: 2.5 $\mu\text{g}/\text{plate}$ (TA 1535, TA 1537, TA 98, TA 100),
10 $\mu\text{g}/\text{plate}$ (WP2 uvrA)

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is sufficient evidence of biological stability.

8.3 Test System

8.3.1 Characterisation of the *Salmonella typhimurium* Strains and *E. coli* Strain

The histidine dependent strains are derived from *S. typhimurium* strain LT2 through a mutation in the histidine locus. Additionally due to the "deep rough" (*rfa*-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "*uvrB*-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker (6).

Strain WP2 (4) and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (*Trp*⁺) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the *uvrA* derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarised the mutations of the TA strains and the *E. coli* strain, used in this study can be described as follows:

<i>Salmonella typhimurium</i>		
Strains	Genotype	Type of mutations indicated
TA 1537	his C 3076; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻	frame shift mutations
TA 98	his D 3052; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻ ; R-factor	" "
TA 1535	his G 46; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻	base-pair substitutions
TA 100	his G 46; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻ ; R-factor	" "
<i>Escherichia coli</i>		
WP2 <i>uvrA</i>	<i>trp</i> ⁻ ; <i>uvrA</i> ⁻	base-pair substitutions and others

Regular checking of the properties of the strains regarding the membrane permeability and ampicillin resistance as well as spontaneous mutation rates is performed in Harlan CCR according to B. Ames et al. (1) and D. Maron and B. Ames (6). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and WP2 *uvrA* were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

8.3.2 Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

8.3.3 Precultures

From the thawed ampoules of the strains 0.5 mL suspension was transferred into 250 mL Erlenmeyer flasks containing 20 mL nutrient medium. A solution of 20 μ L ampicillin (25 μ g/mL) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37° C. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8 - 10^9 cells/mL).

8.3.4 Selective Agar

The plates with the selective agar were obtained from E. Merck, D-64293 Darmstadt.

8.3.5 Overlay Agar

The overlay agar contains per litre:

for Salmonella strains:

6.0 g MERCK Agar Agar*
6.0 g NaCl*
10.5mg L-Histidine· HCl· H₂O*
12.2mg Biotin*

for Escherichia coli:

6.0 g MERCK Agar Agar*
6.0 g NaCl*
2.5 mg Tryptophan*

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121 °C in an autoclave.

8.4 Mammalian Microsomal Fraction S9 Mix

The bacteria used in this assay do not possess the enzyme systems which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

8.4.1 S9 (Preparation by Harlan C C R)

Phenobarbital/ β -Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU, Harlan Laboratories GmbH, 33178 Borcheln, Germany), weight approx. 220 - 320 g induced by applications of 80 mg/kg b.w. Phenobarbital i.p. (Desitin; D-22335 Hamburg) and β -Naphthoflavone p.o. (Aldrich, D-89555 Steinheim) each on three consecutive days. The livers are prepared 24 hours after the last treatment. The S9 fractions are produced by dilution of the liver homogenate with a KCl solution (1+3) followed by centrifugation at 9000 g. Aliquots of the supernatant are frozen and stored in ampoules at -80 °C. Small numbers of the ampoules can be kept at -20 °C for up to one week. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration in the S9 preparation was 30.7 mg/mL (lot no. R 051208).

8.4.2 S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10% v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM MgCl_2
33 mM KCl
5 mM Glucose-6-phosphate
4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(1).

8.5 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with strains TA 1535, TA 1537, TA 98, TA 100, and WP2 uvrA. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).

Toxicity of the test item results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I, if the following criteria are met:

Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

8.6 Dose Selection

In the pre-experiment the concentration range of the test item was 3 - 5000 µg/plate. The pre-experiment is reported as experiment I. Since no relevant toxic effects were observed 5000 µg/plate were chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

33; 100; 333; 1000; 2500; and 5000 µg/plate

8.7 Experimental Performance

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level (solvent or reference mutagen solution (positive control)),
- 500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark (2).

8.8 Data Recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB9 7BN, UK) with the software program Ames Study Manager. The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to precipitation of the test item the revertant colonies were partly counted manually.

8.9 Acceptability of the Assay

The *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

8.10 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 uvrA) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed (3).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (2).

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

8.11 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

9 DISCUSSION OF RESULTS

The test item [] was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls were tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in both experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with [] at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

10 REFERENCES

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11 DISTRIBUTION OF THE REPORT

Sponsor	2 · (copy)
Study Director	1 · (original)

12 SUMMARY OF RESULTS

12.1 Summary of Results Pre-Experiment and Experiment I

Study Name: 1228602
 Experiment: 1228602 VV Plate
 Assay Conditions:

Study Code: Harlan-CCR 1228602
 Date Plated: 09/02/2009
 Date Counted: 12/02/2009

Metabolic Activation	Test Group	Dose Level ($\mu\text{g}/\text{plate}$)	Revertant Colony Counts (Mean \pm SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	DMSO Untreated		15 \pm 3	11 \pm 3	29 \pm 2	142 \pm 7	55 \pm 1
			21 \pm 3	8 \pm 1	28 \pm 4	144 \pm 6	55 \pm 8
		3 μg	20 \pm 4	12 \pm 2	26 \pm 4	136 \pm 6	49 \pm 2
		10 μg	15 \pm 4	10 \pm 3	25 \pm 4	128 \pm 19	54 \pm 18
		33 μg	18 \pm 3	8 \pm 2	28 \pm 1	138 \pm 12	52 \pm 6
		100 μg	19 \pm 2	10 \pm 3	23 \pm 5	147 \pm 6	49 \pm 1
		333 μg	20 \pm 4	8 \pm 1	24 \pm 5	143 \pm 3	58 \pm 9
		1000 μg	18 \pm 3	10 \pm 2	29 \pm 6	134 \pm 11	59 \pm 8
		2500 μg	19 \pm 2 ^P	11 \pm 2 ^P	28 \pm 3 ^P	128 \pm 13 ^P	49 \pm 6 ^P
		5000 μg	16 \pm 5 ^{PM}	8 \pm 1 ^{PM}	19 \pm 1 ^{PM}	121 \pm 5 ^{PM}	50 \pm 5 ^{PM}
	NaN3	10 μg	1966 \pm 63			2019 \pm 63	
	4-NOPD	10 μg			439 \pm 45		
	4-NOPD	50 μg		92 \pm 1			
	MMS	3.0 μL					1390 \pm 90
With Activation	DMSO Untreated		16 \pm 1	11 \pm 3	28 \pm 5	161 \pm 12	62 \pm 7
			15 \pm 2	12 \pm 4	32 \pm 3	155 \pm 2	63 \pm 7
		3 μg	17 \pm 2	13 \pm 4	32 \pm 5	163 \pm 11	62 \pm 10
		10 μg	17 \pm 4	9 \pm 3	30 \pm 2	157 \pm 4	61 \pm 8
		33 μg	19 \pm 3	11 \pm 3	28 \pm 4	153 \pm 4	61 \pm 11
		100 μg	18 \pm 2	14 \pm 2	26 \pm 5	154 \pm 5	63 \pm 9
		333 μg	19 \pm 3	11 \pm 1	30 \pm 2	167 \pm 7	57 \pm 7
		1000 μg	21 \pm 2	13 \pm 4	31 \pm 3	155 \pm 6	58 \pm 3
		2500 μg	20 \pm 2 ^P	13 \pm 3 ^P	27 \pm 1 ^P	140 \pm 7 ^P	58 \pm 1 ^P
		5000 μg	17 \pm 3 ^{PM}	7 \pm 2 ^{PM}	22 \pm 3 ^{PM}	128 \pm 6 ^{PM}	56 \pm 3 ^{PM}
	2-AA	2.5 μg	379 \pm 12	265 \pm 13	1859 \pm 56	2699 \pm 109	
	2-AA	10.0 μg					237 \pm 42

Key to Positive Controls

NaN3 sodium azide
 2-AA 2-aminoanthracene
 4-NOPD 4-nitro-o-phenylene-diamine
 MMS methyl methane sulfonate

Key to Plate Postfix Codes

P Precipitate
 M Manual count

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12.2 Summary of Results Experiment II

Study Name: 1228602
Experiment: 1228602 HV2 Pre
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 20/02/2009
Date Counted: 23/02/2009

Metabolic Activation	Test Group	Dose Level ($\mu\text{g}/\text{plate}$)	Revertant Colony Counts (Mean \pm SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	DMSO Untreated		19 \pm 0	13 \pm 4	25 \pm 4	126 \pm 9	52 \pm 8
			18 \pm 2	10 \pm 3	26 \pm 9	142 \pm 18	51 \pm 12
		33 μg	19 \pm 4	11 \pm 3	26 \pm 8	122 \pm 13	49 \pm 14
		100 μg	15 \pm 3	12 \pm 2	26 \pm 7	120 \pm 4	48 \pm 8
		333 μg	18 \pm 3	8 \pm 2	24 \pm 2	117 \pm 14	48 \pm 8
		1000 μg	12 \pm 1	10 \pm 3	22 \pm 4	121 \pm 13	49 \pm 8
		2500 μg	15 \pm 1 ^P	12 \pm 5 ^P	27 \pm 1 ^P	121 \pm 13 ^P	56 \pm 0 ^P
		5000 μg	11 \pm 1 ^{PM}	9 \pm 3 ^{PM}	22 \pm 1 ^{PM}	111 \pm 9 ^{PM}	46 \pm 6 ^{PM}
	NaN3	10 μg	2008 \pm 41			2037 \pm 39	
	4-NOPD	10 μg			415 \pm 36		
	4-NOPD	50 μg		91 \pm 3			
	MMS	3.0 μL					353 \pm 73
With Activation	DMSO Untreated		19 \pm 4	10 \pm 2	37 \pm 6	152 \pm 17	56 \pm 14
			18 \pm 1	13 \pm 5	30 \pm 5	170 \pm 20	49 \pm 5
		33 μg	16 \pm 6	12 \pm 2	37 \pm 3	144 \pm 10	53 \pm 9
		100 μg	17 \pm 4	9 \pm 3	36 \pm 7	157 \pm 8	56 \pm 2
		333 μg	16 \pm 3	12 \pm 2	33 \pm 2	151 \pm 4	58 \pm 11
		1000 μg	15 \pm 4	10 \pm 0	38 \pm 9	145 \pm 16	57 \pm 6
		2500 μg	18 \pm 6 ^P	11 \pm 2 ^P	33 \pm 2 ^P	133 \pm 4 ^P	55 \pm 2 ^P
		5000 μg	14 \pm 2 ^{PM}	12 \pm 2 ^{PM}	26 \pm 1 ^{PM}	151 \pm 12 ^{PM}	48 \pm 8 ^{PM}
	2-AA	2.5 μg	304 \pm 17	204 \pm 14	1691 \pm 133	2358 \pm 133	
	2-AA	10.0 μg					203 \pm 8

Key to Positive Controls

NaN3 sodium azide
2-AA 2-aminoanthracene
4-NOPD 4-nitro-o-phenylene-diamine
MMS methyl methane sulfonate

Key to Plate Postfix Codes

P Precipitate
M Manual count

228

13 HISTORICAL CONTROL DATA

These data represent the laboratory's historical control data from January 2008 until October 2008 representing approx. 600 experiments (WP2 uvrA the historical data are based on approx. 300 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	17	5.17	9	39	21	5.82	8	41
	Negative control	17	5.33	9	38	20	6.23	10	46
	Positive control	2024	315.78	1041	3138	294	140.02	102	945
TA1537	Solvent control	13	3.12	6	25	17	3.90	9	35
	Negative control	13	3.38	5	26	18	4.05	8	31
	Positive control	116	30.52	68	407	204	69.54	72	454
TA 98	Solvent control	30	5.59	13	59	39	6.34	20	60
	Negative control	31	5.45	16	55	39	6.53	19	59
	Positive control	489	169.76	211	1694	1455	463.01	200	3553
TA 100	Solvent control	130	18.79	89	224	155	22.54	92	218
	Negative control	139	17.30	93	205	147	21.78	92	234
	Positive control	2160	342.67	588	3379	1839	621.27	404	3868
WP2uvrA	Solvent control	49	8.02	33	82	60	8.76	34	89
	Negative control	49	8.58	30	79	60	8.71	31	84
	Positive control	986	777.44	187	2367	414	441.93	164	1597

Mean = mean value of revertants/plate

SD = standard deviation

Min = minimal value/Max = maximal value

14 ANNEX: TABLES OF RESULTS (8 PAGES)

Pre-Experiment and Experiment I: 1228602 VV Plate Incorporation (4 pages)

Experiment II: 1228602 Pre-Incubation (4 pages)

Study Name: 1228602
Experiment: 1228602 VV Plate
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 09/02/2009
Date Counted: 12/02/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535		3 µg	19.7	4.2	1.3	23, 21, 15
		10 µg	15.3	4.0	1.0	13, 20, 13
		33 µg	18.3	3.2	1.2	16, 17, 22
		100 µg	18.7	2.3	1.2	16, 20, 20
		333 µg	19.7	4.2	1.3	15, 23, 21
		1000 µg	18.3	3.2	1.2	17, 16, 22
		2500 µg	18.7	1.5	1.2	19 P, 20 P, 17 P
		5000 µg	16.0	5.0	1.0	16 P M, 11 P M, 21 P M
	DMSO		15.3	3.2		19, 13, 14
	Untreated Control		21.0	3.5		23, 17, 23
TA 1537		3 µg	12.0	2.0	1.1	14, 10, 12
		10 µg	10.3	3.2	0.9	8, 9, 14
		33 µg	7.7	1.5	0.7	8, 9, 6
		100 µg	10.3	3.2	0.9	14, 9, 8
		333 µg	7.7	0.6	0.7	8, 8, 7
		1000 µg	9.7	2.1	0.9	12, 8, 9
		2500 µg	11.0	1.7	1.0	13 P, 10 P, 10 P
		5000 µg	7.7	1.2	0.7	7 P M, 9 P M, 7 P M
	DMSO		11.0	3.5		7, 13, 13
	Untreated Control		7.7	0.6		8, 7, 8
TA 98		3 µg	26.3	3.5	0.9	23, 30, 26
		10 µg	25.0	4.4	0.9	20, 27, 28
		33 µg	28.3	1.2	1.0	27, 29, 29
		100 µg	23.0	4.6	0.8	19, 22, 28
		333 µg	24.3	5.1	0.8	20, 23, 30
		1000 µg	28.7	5.7	1.0	35, 27, 24
		2500 µg	28.0	2.6	1.0	30 P, 29 P, 25 P
		5000 µg	19.3	0.6	0.7	19 P M, 20 P M, 19 P M
	DMSO		29.0	2.0		27, 31, 29
	Untreated Control		28.0	3.6		24, 31, 29
TA 100		3 µg	135.7	5.5	1.0	136, 130, 141
		10 µg	127.7	19.0	0.9	127, 109, 147
		33 µg	138.0	11.8	1.0	148, 125, 141
		100 µg	146.7	5.9	1.0	151, 140, 149
		333 µg	143.3	3.2	1.0	147, 142, 141
		1000 µg	134.0	11.3	0.9	140, 141, 121
		2500 µg	128.3	12.7	0.9	143 P, 122 P, 120 P
		5000 µg	121.3	4.9	0.9	119 P M, 127 P M, 118 P M
	DMSO		142.3	7.1		141, 136, 150
	Untreated Control		144.0	6.1		140, 151, 141

Key to Plate Postfix Codes

P Precipitate
M Manual count

Study Name: 1228602
Experiment: 1228602 VV Plate
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 09/02/2009
Date Counted: 12/02/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA		3 µg	49.3	1.5	0.9	48, 51, 49
		10 µg	54.3	18.5	1.0	65, 65, 33
		33 µg	52.3	6.1	1.0	51, 59, 47
		100 µg	49.0	1.0	0.9	50, 48, 49
		333 µg	58.3	9.3	1.1	66, 61, 48
		1000 µg	58.7	7.5	1.1	63, 63, 50
		2500 µg	48.7	5.7	0.9	55 P, 47 P, 44 P
		5000 µg	49.7	5.1	0.9	54 P M, 44 P M, 51 P M
	DMSO		55.0	1.0		55, 56, 54
	Untreated Control		55.0	8.0		55, 63, 47
TA 1535	NaN3	10 µg	1966.0	62.6	128.2	1906, 2031, 1961
TA 1537	4-NOPD	50 µg	92.3	0.6	8.4	93, 92, 92
TA 98	4-NOPD	10 µg	438.7	45.4	15.1	449, 478, 389
TA 100	NaN3	10 µg	2019.3	63.3	14.2	2041, 2069, 1948
WP2 uvrA	MMS	3.0 µL	1389.7	89.6	25.3	1457, 1424, 1288

Key to Positive Controls

NaN3 sodium azide
4-NOPD 4-nitro-o-phenylene-diamine
MMS methyl methane sulfonate

Key to Plate Postfix Codes

P Precipitate
M Manual count

Study Name: 1228602
Experiment: 1228602 VV Plate
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 09/02/2009
Date Counted: 12/02/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535		3 µg	16.7	2.1	1.0	15, 16, 19
		10 µg	16.7	4.0	1.0	19, 19, 12
		33 µg	18.7	2.9	1.2	17, 22, 17
		100 µg	17.7	2.1	1.1	20, 17, 16
		333 µg	19.0	3.0	1.2	16, 22, 19
		1000 µg	21.3	1.5	1.3	20, 21, 23
		2500 µg	20.0	1.7	1.3	22 P, 19 P, 19 P
		5000 µg	17.3	3.2	1.1	21 P M, 15 P M, 16 P M
		DMSO	16.0	1.0		16, 15, 17
	Untreated Control		15.3	2.1		16, 13, 17
TA 1537		3 µg	13.3	3.8	1.2	9, 16, 15
		10 µg	9.3	2.5	0.8	7, 12, 9
		33 µg	10.7	3.2	1.0	12, 7, 13
		100 µg	14.0	1.7	1.3	12, 15, 15
		333 µg	10.7	1.2	1.0	10, 10, 12
		1000 µg	13.3	4.0	1.2	17, 14, 9
		2500 µg	13.3	2.9	1.2	15 P, 15 P, 10 P
		5000 µg	6.7	1.5	0.6	7 P M, 5 P M, 8 P M
		DMSO	11.0	2.6		14, 9, 10
	Untreated Control		11.7	3.8		9, 10, 16
TA 98		3 µg	32.0	5.3	1.1	36, 34, 26
		10 µg	30.0	1.7	1.1	31, 28, 31
		33 µg	28.0	4.4	1.0	31, 30, 23
		100 µg	25.7	4.7	0.9	22, 31, 24
		333 µg	29.7	1.5	1.1	31, 30, 28
		1000 µg	31.3	2.9	1.1	33, 33, 28
		2500 µg	27.3	1.2	1.0	28 P, 26 P, 28 P
		5000 µg	21.7	2.5	0.8	22 P M, 19 P M, 24 P M
		DMSO	28.0	5.0		28, 33, 23
	Untreated Control		32.3	3.1		33, 35, 29
TA 100		3 µg	163.0	10.6	1.0	159, 175, 155
		10 µg	157.0	4.4	1.0	162, 155, 154
		33 µg	153.3	4.0	1.0	151, 151, 158
		100 µg	154.3	4.7	1.0	149, 156, 158
		333 µg	167.0	7.2	1.0	169, 159, 173
		1000 µg	155.0	6.1	1.0	162, 151, 152
		2500 µg	140.3	6.7	0.9	136 P, 137 P, 148 P
		5000 µg	128.3	6.0	0.8	129 P M, 134 P M, 122 P M
		DMSO	161.3	12.2		164, 172, 148
	Untreated Control		155.3	1.5		155, 157, 154

Key to Plate Postfix Codes

P Precipitate
M Manual count

Study Name: 1228602
Experiment: 1228602 VV Plate
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 09/02/2009
Date Counted: 12/02/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA		3 µg	62.3	10.1	1.0	56, 74, 57
		10 µg	61.0	7.9	1.0	70, 55, 58
		33 µg	61.3	10.8	1.0	66, 69, 49
		100 µg	63.3	9.0	1.0	72, 64, 54
		333 µg	57.3	7.1	0.9	65, 51, 56
		1000 µg	57.7	3.1	0.9	61, 55, 57
		2500 µg	58.0	1.0	0.9	58 P, 57 P, 59 P
		5000 µg	56.0	3.5	0.9	52 P M, 58 P M, 58 P M
		DMSO	61.7	7.2		70, 57, 58
	Untreated Control		62.7	7.1		69, 64, 55
TA 1535	2-AA	2.5 µg	378.7	12.0	23.7	367, 391, 378
TA 1537	2-AA	2.5 µg	265.3	12.7	24.1	263, 254, 279
TA 98	2-AA	2.5 µg	1859.3	56.2	66.4	1832, 1924, 1822
TA 100	2-AA	2.5 µg	2698.7	108.5	16.7	2824, 2636, 2636
WP2 uvrA	2-AA	10.0 µg	237.3	42.0	3.8	189, 258, 265

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate
M Manual count

Study Name: 1228602
Experiment: 1228602 HV2 Pre
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 20/02/2009
Date Counted: 23/02/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535		33 µg	19.0	3.6	1.0	20, 15, 22
		100 µg	15.3	2.9	0.8	12, 17, 17
		333 µg	18.3	2.9	1.0	15, 20, 20
		1000 µg	12.3	0.6	0.6	13, 12, 12
		2500 µg	15.0	1.0	0.8	15 P, 14 P, 16 P
		5000 µg	11.0	1.0	0.6	10 P M, 11 P M, 12 P M
		DMSO	19.0	0.0		19, 19, 19
		Untreated Control	17.7	2.1		16, 17, 20
TA 1537		33 µg	10.7	2.9	0.8	9, 9, 14
		100 µg	11.7	2.3	0.9	13, 13, 9
		333 µg	8.0	1.7	0.6	7, 10, 7
		1000 µg	10.3	2.5	0.8	13, 8, 10
		2500 µg	12.3	4.6	1.0	15 P, 7 P, 15 P
		5000 µg	9.3	3.2	0.7	7 P M, 8 P M, 13 P M
		DMSO	12.7	4.0		15, 8, 15
		Untreated Control	10.0	3.0		13, 7, 10
TA 98		33 µg	25.7	7.5	1.0	30, 30, 17
		100 µg	26.3	6.5	1.0	26, 33, 20
		333 µg	24.3	1.5	1.0	23, 24, 26
		1000 µg	22.0	3.6	0.9	26, 21, 19
		2500 µg	27.3	0.6	1.1	27 P, 27 P, 28 P
		5000 µg	21.7	1.2	0.9	21 P M, 21 P M, 23 P M
		DMSO	25.3	4.0		30, 23, 23
		Untreated Control	26.0	8.9		29, 16, 33
TA 100		33 µg	121.7	13.1	1.0	134, 108, 123
		100 µg	120.3	3.8	1.0	116, 122, 123
		333 µg	117.0	14.0	0.9	101, 127, 123
		1000 µg	120.7	12.7	1.0	128, 106, 128
		2500 µg	121.0	13.0	1.0	121 P, 134 P, 108 P
		5000 µg	111.3	8.7	0.9	109 P M, 104 P M, 121 P M
		DMSO	125.7	9.3		123, 118, 136
		Untreated Control	141.7	17.8		134, 162, 129
WP2 uvrA		33 µg	48.7	13.9	0.9	45, 64, 37
		100 µg	47.7	8.0	0.9	47, 40, 56
		333 µg	48.3	7.6	0.9	40, 50, 55
		1000 µg	49.3	8.3	1.0	56, 40, 52
		2500 µg	56.0	0.0	1.1	56 P, 56 P, 56 P
		5000 µg	45.7	5.7	0.9	44 P M, 41 P M, 52 P M
		DMSO	51.7	7.5		59, 52, 44
		Untreated Control	51.3	11.6		57, 38, 59

Key to Plate Postfix Codes

P Precipitate
M Manual count

Study Name: 1228602
Experiment: 1228602 HV2 Pre
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 20/02/2009
Date Counted: 23/02/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	NaN3	10 µg	2008.0	41.0	105.7	2048, 1966, 2010
TA 1537	4-NOPD	50 µg	91.3	2.9	7.2	88, 93, 93
TA 98	4-NOPD	10 µg	414.7	35.9	16.4	421, 447, 376
TA 100	NaN3	10 µg	2037.0	39.0	16.2	2027, 2080, 2004
WP2 uvrA	MMS	3.0 µL	352.7	73.2	6.8	286, 341, 431

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 1228602
 Experiment: 1228602 HV2 Pre
 Assay Conditions:

Study Code: Harlan-CCR 1228602
 Date Plated: 20/02/2009
 Date Counted: 23/02/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535		33 µg	15.7	5.5	0.8	13, 12, 22
		100 µg	17.0	4.0	0.9	13, 17, 21
		333 µg	16.0	2.6	0.9	19, 14, 15
		1000 µg	14.7	3.8	0.8	12, 19, 13
		2500 µg	18.3	6.0	1.0	12 P, 24 P, 19 P
		5000 µg	14.3	2.3	0.8	13 P M, 13 P M, 17 P M
		DMSO	18.7	3.8		17, 23, 16
		Untreated Control	17.7	1.2		17, 19, 17
TA 1537		33 µg	11.7	1.5	1.2	10, 12, 13
		100 µg	9.3	2.5	1.0	12, 7, 9
		333 µg	12.0	1.7	1.2	10, 13, 13
		1000 µg	10.0	0.0	1.0	10, 10, 10
		2500 µg	11.0	1.7	1.1	10 P, 13 P, 10 P
		5000 µg	11.7	2.1	1.2	10 P M, 11 P M, 14 P M
		DMSO	9.7	2.1		9, 8, 12
		Untreated Control	13.0	5.3		17, 7, 15
TA 98		33 µg	37.3	3.2	1.0	36, 41, 35
		100 µg	36.0	7.0	1.0	33, 44, 31
		333 µg	33.3	2.1	0.9	35, 31, 34
		1000 µg	38.3	9.0	1.0	28, 44, 43
		2500 µg	33.3	2.1	0.9	34 P, 31 P, 35 P
		5000 µg	26.0	1.0	0.7	26 P M, 25 P M, 27 P M
		DMSO	36.7	6.4		44, 33, 33
		Untreated Control	30.3	5.1		29, 36, 26
TA 100		33 µg	143.7	9.6	0.9	154, 142, 135
		100 µg	157.3	8.0	1.0	158, 165, 149
		333 µg	151.3	4.0	1.0	149, 149, 156
		1000 µg	144.7	15.6	1.0	161, 130, 143
		2500 µg	133.0	4.0	0.9	133 P, 129 P, 137 P
		5000 µg	151.0	11.5	1.0	140 P M, 150 P M, 163 P M
		DMSO	151.7	17.2		136, 149, 170
		Untreated Control	170.3	19.5		170, 190, 151
WP2 uvrA		33 µg	53.3	9.0	1.0	59, 58, 43
		100 µg	55.7	1.5	1.0	57, 54, 56
		333 µg	58.0	11.0	1.0	69, 47, 58
		1000 µg	56.7	5.9	1.0	59, 50, 61
		2500 µg	55.3	1.5	1.0	57 P, 54 P, 55 P
		5000 µg	48.3	7.6	0.9	55 P M, 50 P M, 40 P M
		DMSO	55.7	14.0		69, 41, 57
		Untreated Control	49.3	5.0		44, 50, 54

Key to Plate Postfix Codes

P Precipitate
 M Manual count

Study Name: 1228602
Experiment: 1228602 HV2 Pre
Assay Conditions:

Study Code: Harlan-CCR 1228602
Date Plated: 20/02/2009
Date Counted: 23/02/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	2-AA	2.5 µg	304.3	16.8	16.3	315, 313, 285
TA 1537	2-AA	2.5 µg	204.0	13.7	21.1	192, 201, 219
TA 98	2-AA	2.5 µg	1690.7	133.1	46.1	1571, 1667, 1834
TA 100	2-AA	2.5 µg	2358.3	132.6	15.5	2378, 2480, 2217
WP2 uvrA	2-AA	10.0 µg	203.3	8.1	3.7	208, 194, 208

Key to Positive Controls

2-AA	2-aminoanthracene
------	-------------------

STUDY NUMBER 1228601

IN VITRO

CHROMOSOME ABERRATION TEST

IN CHINESE HAMSTER V79 CELLS

WITH

[]

REPORT

Date of the Report:

July 01, 2009

1 COPY OF THE GLP CERTIFICATE

HESSEN



Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility

☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH

Harlan Cytotest Cell Research GmbH

In den Leppsteinswiesen 19

64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise

(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften

2 Toxicity studies

3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)

3 Mutagenicity studies

6 Prüfungen zur Bestimmung von Rückständen

6 Residues

8 Analytische Prüfungen an biologischen Materialien

8 Analytical studies on biological materials

15.08. und 27. – 29.10.2008

Datum der Inspektion/Date of Inspection

(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Th. Zimmermann, Referent, Wiesbaden, den 30. März 2009

(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)



Hess. Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden

(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

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3 PREFACE

3.1 General

Title: *In vitro* Chromosome Aberration Test
in Chinese Hamster V79 Cells
with []

Sponsor: Cheil Industries Inc.
Sunghee Ahn
(437-711) 332-2, Gocheon-dong
Uiwang-si, Gyeonggi-do, Korea

Study Monitor: Ms. Jin-Sil Bak
Chemtopia Co. Ltd
(137-876) No. 1407, Leaders Building
1599-11, Seocho-dong
Seocho-gu
Seoul, Korea

Test Facility: Harlan Cytotest Cell Research GmbH (Harlan CCR)
In den Leppsteinswiesen 19
64380 Rossdorf
Germany

Contracting Institute: Harlan Laboratories Ltd.
4452 Itingen
Switzerland

Reference Number: C28602

3.2 Responsibilities

Study Director: Dr. Susanne Bohnenberger

Deputy Study Director: Dr. Caroline Hall (from February 09, 2009)

Management: Dr. Wolfgang Völkner

Head of
Quality Assurance Unit: Frauke Hermann

3.3 Schedule

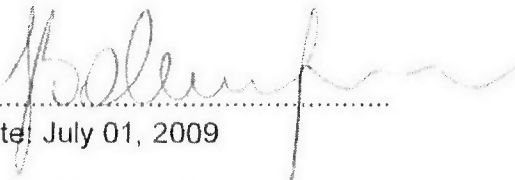
Experimental Starting Date: January 28, 2009

Experimental Completion Date: June 18, 2009

3.4 Project Staff Signatures

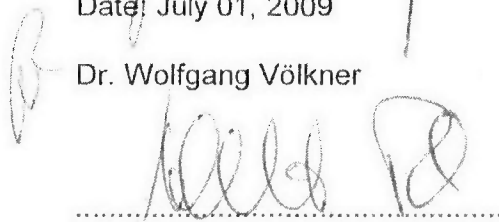
Study Director

Dr. Susanne Bohnenberger


Date: July 01, 2009

Management

Dr. Wolfgang Völkner


Date: July 01, 2009

Dr. A. Poth

3.5 Good Laboratory Practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002, and amended version dated July 02, 2008 ("BGBl.", p. 1146).

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

3.6 Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline No. 473 "*In vitro* Mammalian Chromosome Aberration Test".

Commission Regulation (EC) No. 440/2008, B10: "Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test", dated May 30, 2008.

3.7 Archiving

Harlan CCR will archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

Microscopic slides will be archived for at least 12 years.

No data will be discarded without the sponsor's consent.

3.8 Deviations from the Study Plan

There were no deviations from the study plan.

4 STATEMENT OF COMPLIANCE

Study Number: 1228601

Test Item: []

Study Director: Dr. Susanne Bohnenberger

Title: *In vitro* Chromosome Aberration Test
in Chinese Hamster V79 Cells
with []

This study performed in the test facility of Harlan CCR was conducted in compliance with Good Laboratory Practice Regulations:

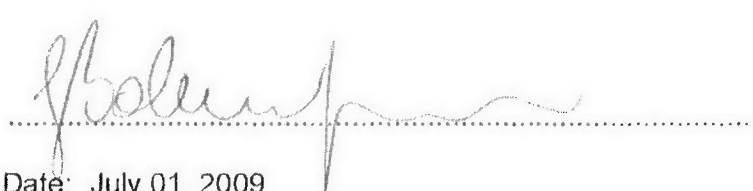
"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002, and amended version dated July 02, 2008 ("BGBl.", p. 1146).

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

Harlan CCR
Dr. Susanne Bohnenberger


Date: July 01, 2009

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1228601

Test Item:

[]

Study Director: Dr. Susanne Bohnenberger

Title: *In vitro* Chromosome Aberration Test
in Chinese Hamster V79 Cells
with []

The general facilities and activities of Harlan CCR are inspected periodically and the results are reported to the responsible person and the Management.

Study procedures were inspected periodically. The study plan and this interim report were audited by the Quality Assurance Unit. The dates are given below.

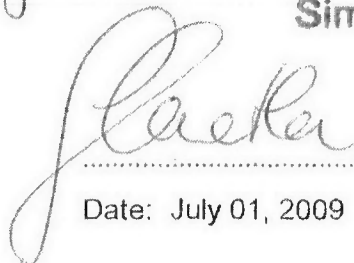
Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	December 12, 2008	December 12, 2008
1 st Amendment to Study Plan:	April 30, 2009 Mai 22, 2009	April 30, 2009 Mai 22, 2009
Process Inspection test performance:	February 19, 2009 March 06, 2009	February 19, 2009 March 06, 2009
Interim Report:	May 25, 2009	May 25, 2009
Report:	June 29, 2009	June 29, 2009

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit

for Frauke Hermann

Simone Günther



Date: July 01, 2009

6 SUMMARY OF RESULTS

The test item [] suspended in DMSO, was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in vitro* in two independent experiments. The following study design was performed:

	Without S9 mix		With S9 mix	
	Exp. I	Exp. II	Exp. I	Exp. II
Exposure period	4 hours	18 hours	4 hours	4 hours
Recovery	14 hours	-	14 hours	14 hours
Preparation interval	18 hours	18 hours	18 hours	18 hours

In each experimental group two parallel cultures were set up. 100 metaphases per culture were evaluated for structural chromosome aberrations.

The highest applied concentration (5000 µg/mL) was chosen with respect to the current OECD Guideline 473.

Dose selection for the cytogenetic experiments was performed considering the toxicity data and the occurrence of precipitation. The chosen treatment concentrations are described in chapter 8.6 (page 16). The evaluated experimental points and the results are summarised in Table 1 (page 23).

In Experiment I in the absence and presence of S9 mix no cytotoxicity was observed up to the highest evaluable concentrations. Higher concentrations could not be evaluated for genotoxicity due to strong test item precipitation. However, the mitotic indices were reduced below 60 % of control after treatment with 5000.0 µg/mL in the absence of S9 mix and with 2500 µg/mL and above in the presence of S9 mix.

In Experiment II in the absence of S9 mix the mitotic indices were reduced to 60 % of control at the highest evaluable concentration. In the presence of S9 mix no cytotoxicity was observed up to the highest applied concentration.

No clastogenicity was observed at the concentrations evaluated either with or without metabolic activation.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations.

6.1 Conclusion

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) *in vitro*.

Therefore, [] is considered to be non-clastogenic in this chromosome aberration test in the absence and presence of metabolic activation, when tested up to precipitating or moderate cytotoxic concentrations.

7 INTRODUCTION

According to national and international acts chemicals have to be tested before introduction to the market for a possible hazard to humans and the environment. Genotoxicity studies provide important information for the assessment of the mutagenic potential of these substances (1, 4). The *in vitro* Chromosome Aberration Test performed in this study is an essential part of genotoxicity test batteries of substances.

This *in vitro* test is a test for the detection of structural chromosomal aberrations. Such aberrations are frequently lethal to the damaged cells (8, 10). However, cytogenetic damage in somatic cells is an indicator of a potential to induce subtle chromosomal damage that may be compatible with cell division. Similar damage induced in germ cells may lead to heritable cytogenetic abnormalities. Heritable cytogenetic abnormalities are known to have deleterious effects in man, e.g. induction of neoplastic events or birth defects. Also, chromosome abnormalities in somatic cells may become one of the reasons why a transformed cell population may develop into cancer.

Chromosome aberrations are generally evaluated in first post treatment mitoses. The majority of chemical mutagens induced structural aberrations are of the chromatid type, but chromosome type aberrations also occur.

For treatment, cell populations should be in exponential growth to guarantee that there are cells in all stages of the cell cycle (i.e. an asynchronous population). Since the normal cell cycle time is 13 - 15 hours (see page 14) and the guidelines require fixation times of about 1.5-fold of the normal cell cycle, a fixation time of around 18 hours is appropriate. Due to the limited capacity of the V79 cells for metabolic activation of potential mutagens an exogenous metabolic activation system is included (7).

To validate the test reference mutagens were tested in parallel to the test item.

7.1 Aims of the Study

This *in vitro* test was performed to assess the potential of [] to induce structural chromosome aberrations. Evaluation of cytogenetic damage induced in V79 cells (cell line from the lung of the Chinese Hamster) in the absence and the presence of metabolic activation was performed in two independent experiments at one preparation interval (18 hours).

8 MATERIALS AND METHODS

8.1 Test Item

Internal Test Item Number: S 961311

The test item and the information concerning the test item were provided by the sponsor.

Identity:

Chemical Name:

Batch No.:

20081010

Molecular Weight:

Purity:

>99 %

Stability in Solvent:

Not indicated by the sponsor

Storage:

At room temperature

Expiration Date:

January 08, 2011

On the day of the experiment (immediately before treatment), the test item was suspended in DMSO (E. MERCK, 64293 Darmstadt, Germany; purity 99.5 %). The final concentration of DMSO in the culture medium will be 0.5 % (v/v). The solvent was chosen to its solubility properties and its relative non-toxicity to the cell cultures.

8.2 Controls

8.2.1 Solvent Controls

Concurrent solvent controls (DMSO) were performed.

Name: DMSO; Dimethyl sulfoxid
Supplier: E. MERCK, 64293 Darmstadt, Germany
Purity: 99.5 %
Lot no.: K38528031821
K39250731847

8.2.2 Positive Control Substances

Without metabolic activation

Name: EMS; ethylmethane sulfonate
Supplier: ACROS ORGANICS, 2440 Geel, Belgium
Purity: $\geq 98\%$
Lot no.: A0246840
Expiration Date: April 2009
Dissolved in: Nutrient medium
Final Concentration: 500 and 1000 $\mu\text{g/mL}$ (4.0 and 8.0 mM)

Solutions were prepared on the day of experiment. The stability of the positive control substance in solution was proven by the mutagenic response in the expected range.

With metabolic activation

Name: CPA; cyclophosphamide
Supplier: Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany
Purity: $\geq 98\%$
Lot no.: 097K1311
Expiration Date: March 2011
Dissolved in: Saline (0.9 % [w/v])
Final Concentration: 1.4 $\mu\text{g/mL}$ (5.0 μM)

The dilutions of the stock solutions were prepared on the day of experiment. The stability of CPA in solution at room temperature is good. At 25 °C only 3.5 % of its potency is lost after 24 hours (6).

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8.3 Test System

8.3.1 Reasons for the Choice of the Cell Line V79

The V79 cell line has been used successfully for many years in *in vitro* experiments. The high proliferation rate (doubling time of clone V79/D3 in stock cultures: 14 hours, determined on April 18, 2008) and a reasonable plating efficiency of untreated cells (as a rule more than 70 %) both necessary for the appropriate performance of the study, support the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.

Lacking metabolic activities of cells under *in vitro* conditions are a disadvantage of tests with cell cultures as many chemicals only develop a mutagenic potential when they are metabolized by the mammalian organism. However, metabolic activation of chemicals can be achieved at least partially by supplementing the cell cultures with liver microsome preparations (S9 mix).

8.3.2 Cell Cultures

Large stocks of the V79 cell line (supplied by Laboratory for Mutagenicity Testing, LMP, Technical University Darmstadt, 64287 Darmstadt, Germany) were stored in liquid nitrogen in the cell bank of Harlan CCR allowing the repeated use of the same cell culture batch in experiments. Before freezing each batch was screened for mycoplasma contamination and checked for karyotype stability. Consequently, the parameters of the experiments remain similar because of standardized characteristics of the cells.

Thawed stock cultures were propagated at 37 °C in 80 cm² plastic flasks (Greiner, 72632 Frickenhausen, Germany). About 5×10^5 cells per flask were seeded into 15 mL of MEM (Minimal Essential Medium; Seromed, 12247 Berlin, Germany) supplemented with 10 % fetal calf serum (FCS; PAA Laboratories GmbH, 35091 Cölbe, Germany). Additionally, the medium was supplemented with 1 % 100x Penicillin/ Streptomycin solution (10.000 Units/mL Penicillin, 10 mg/mL Streptomycin; PAA Laboratories GmbH, 35091 Cölbe, Germany) and 1 % Amphotericin B-solution (250 µg/mL, PAA Laboratories GmbH, 35091 Cölbe, Germany). The cells were sub-cultured twice weekly. The cell cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).

8.4 Mammalian Microsomal Fraction S9 Mix

8.4.1 S9 (Preparation by Harlan CCR)

Phenobarbital/ β -Naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from 8 - 12 weeks old male Wistar rats (HsdCpb:WU, Harlan Laboratories GmbH, 33178 Borcheln, Germany) weight approx. 220 - 320 g induced by intraperitoneal applications of 80 mg/kg b.w. Phenobarbital (Desitin; 22335 Hamburg, Germany) and by peroral administrations of 80 mg/kg b.w. β -Naphthoflavone (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1:3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at -80°C . Small numbers of the ampoules were kept at -20°C for up to one week.

The protein concentration was 35.0 mg/mL (Lot no. 211108) in Experiment I and 35.6 mg/mL (Lot no. 270309) in Experiment II.

8.4.2 S9 Mix

The S9 mix preparation was performed according to Ames et al. (1).

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

8 mM MgCl_2
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath.

8.5 Pre-experiment

A pre-test on cell growth inhibition was performed in order to determine the toxicity of the test item (2), the solubility during exposure and changes in osmolarity and pH value at experimental conditions. The test item was applied up to the maximum concentration mentioned above, unless solubility, pH value or osmolarity influence dose selection. Relatively insoluble substances were tested up to the highest concentration that could be formulated in an appropriate solvent as a homogeneous suspension.

The experimental conditions in this pre-experiment were identical to those required and described below for the mutagenicity assay.

8.6 Dose Selection

The highest concentration used in the cytogenetic experiments was chosen considering the current OECD Guideline for *in vitro* mammalian cytogenetic tests requesting for the top concentration clear toxicity with reduced cell numbers or mitotic indices below 50 % of control, whichever is the lowest concentration, and/or the occurrence of precipitation. In case of non-toxicity the maximum concentration should be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest, if formulation in an appropriate solvent is possible.

In the pre-test 5000 µg/mL of SH-1 was applied as top concentration for treatment of the cultures. Test item concentrations between 19.5 and 5000 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. Precipitation of the test item was observed at 312.5 µg/mL and above after 4 hours treatment time in the absence and presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Dose selection of Experiment II was influenced by the results obtained in Experiment I. For the continuous exposure in the absence of S9 mix 5000 µg/mL and for the 4 hours exposure in the presence of S9 mix 1250 µg/mL were chosen as top test item concentrations.

Doses applied in the Chromosome aberration test with SH-1

Preparation interval	Exposure period	Exp.	Concentration in µg/mL								
			Without S9 mix								
18 hrs	4 hrs	I	19.5	39.1	78.1	156.3	312.5^P	625.0^P	1250.0 ^P	2500.0 ^P	5000.0 ^P
18 hrs	18 hrs	II	19.5	39.1	78.1	156.3^P	312.5^P	625.0 ^P	1250.0 ^P	2500.0 ^P	5000.0 ^P
			With S9 mix								
18 hrs	4 hrs	I	19.5	39.1	78.1	156.3	312.5^P	625.0^P	1250.0 ^P	2500.0 ^P	5000.0 ^P
18 hrs	4 hrs	II	4.9	9.8	19.5	39.1^P	78.1^P	156.3 ^P	312.5 ^P	625.0 ^P	1250.0^P

Evaluated experimental points are shown in bold characters

^P Precipitation occurred at the end of treatment

The cytogenetic evaluation of higher concentrations in the respective intervals (with and without S9 mix) was impossible due to strong test item precipitation, except for Experiment II in the presence of S9 mix.

8.7 Experimental Performance

8.7.1 Seeding of the Cultures

Exponentially growing stock cultures more than 50 % confluent were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then the enzymatic treatment was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5 % (w/v) in Ca-Mg-free salt solution (Invitrogen GIBCO, 76131 Karlsruhe, Germany).

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution. The Ca-Mg-free salt solution was composed as follows (per litre):

NaCl	8000 mg
KCl	200 mg
KH ₂ PO ₄	200 mg
Na ₂ HPO ₄	150 mg

The cells were seeded into Quadriperm dishes (Heraeus, 63450 Hanau, Germany) that contained microscopic slides (at least 2 chambers per dish and test group). In each chamber 1×10^4 - 6×10^4 cells were seeded with regard to the preparation time. The medium was MEM with 10 % FCS (complete medium), 1 % 100x Penicillin/ Streptomycin and 1 % Amphotericin B.

8.7.2 Treatment

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium with 1 % 100x Penicillin/-Streptomycin-solution and 1 % Amphotericin B-solution containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium were used. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium for the remaining culture time.

The "Saline G" solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose x H ₂ O	1100 mg
Na ₂ HPO ₄ x 7 H ₂ O	290 mg
KH ₂ PO ₄	150 mg

pH was adjusted to 7.2.

Exposure period 18 hours

The culture medium of exponentially growing cell cultures was replaced with complete medium (with 10 % FCS, 1 % 100x Penicillin/-Streptomycin-solution, 1 % Amphotericin B-solution) containing different concentrations of the test item without S9 mix. The medium was not changed until preparation of the cells.

All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO₂ (98.5 % air).

8.7.3 Preparation of the Cultures

Colcemid was added (0.2 µg/mL culture medium) to the cultures 15.5 hours after the start of the treatment. The cells on the slides were treated 2.5 hours later, in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3:1 parts, respectively). Per experiment two slides per group were prepared. After preparation the cells were stained with Giemsa (E. MERCK, 64293 Darmstadt, Germany).

8.7.4 Evaluation of Cell Numbers

The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

8.7.5 Analysis of Metaphase Cells

Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik" [5]) using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides.

Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

8.8 Data Recording

The data generated were recorded in the raw data file. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive controls.

8.9 Acceptability of the Test

The chromosome aberration test performed in our laboratory is considered acceptable, if it meets the following criteria:

- a) The number of structural aberrations found in the solvent controls falls within the range of the laboratory's historical control data (see chapter 14.1.1, page 33).
- b) The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data (see chapter 14.1.1, page 33).

8.10 Evaluation of Results

A test item is classified as non-clastogenic if:

- the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data range (see chapter 14.1.1, page 33).

and/or

- no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as clastogenic if:

- the number of induced structural chromosome aberrations is not in the range of the laboratory's historical control data range (see chapter 14.1.1, page 33).

and

- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test (9) ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is valid:

A test item can be classified as aneugenic if:

- the number of induced numerical aberrations is not in the range of the laboratory's historical control data range (see chapter 14.1.2, page 34).

9 RESULTS AND DISCUSSION

The test item [] suspended in DMSO, was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in vitro* in the absence and the presence of metabolic activation by S9 mix.

Two independent experiments were performed. In Experiment I, the exposure period was 4 hours with and without metabolic activation. In Experiment II the exposure period was 18 hours without S9 mix and 4 hours with S9 mix. The chromosomes were prepared 18 hours after start of treatment with the test item.

In each experimental group two parallel cultures were set up. 100 metaphases per culture were evaluated for structural chromosome aberrations.

In Experiment I precipitation of the test item in culture medium was observed after 4 hours treatment with 312.5 µg/mL and above in the absence and presence of S9 mix. In Experiment II after 18 hours continuous treatment precipitation was observed with 156.3 µg/mL and above in the absence of S9 mix and with 39.1 µg/mL and above in the presence of S9 mix after 4 hrs treatment. In both experiments no relevant influence of the test item on the pH value or osmolality was observed (Exp. I: solvent control 381 mOsm, pH 7.2 versus 355 mOsm, pH 7.3 at 5000 µg/mL; Exp. II: solvent control 388 mOsm, pH 7.3 versus 394 mOsm, pH 7.3 at 5000 µg/mL).

In Experiment I no clear toxic effects indicated by reduced mitotic indices or reduced cell numbers of below 50 % of control were observed after treatment up to the highest evaluable concentrations (see Table 2 and 3, page 24 and 25). Higher concentrations could not be evaluated for genotoxicity due to strong test item precipitation. However, the mitotic indices were reduced below 60 % of control after treatment with 5000 µg/mL in the absence of S9 mix and with 2500 µg/mL and above in the presence of S9 mix.

In Experiment II in the absence of S9 mix the mitotic index was reduced to 58.4 % of control after 18 hours continuous treatment at the highest evaluable concentration (312.5 µg/mL). In the presence of S9 mix no cytotoxicity was observed up to the highest applied concentration (see Table 6, page 28).

In both experiments, in the absence and presence of S9 mix, no statistically significant and biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed (see Table 4, 5, 7 and 8, pages 26, 27, 29 and 30). The aberration rates of the cells after treatment with the test item (0.0 - 3.5 % aberrant cells, excluding gaps) were close to the range of the solvent control values (1.5 - 3.5 % aberrant cells, excluding gaps) and within the range of the laboratory's historical solvent control data (0.0 - 4.0 % aberrant cells, excluding gaps).

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the controls.

In both experiments, either EMS (500 or 1000 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

In conclusion, it can be stated that under the experimental conditions reported, the test item [] did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) in the absence and presence of S9 mix, when tested up to precipitating or moderate cytotoxic concentrations.

10 DISTRIBUTION OF THE REPORT

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12 ANNEX I: TABLES OF RESULTS

12.1 Summary of Results

Table 1: Summary of results of the chromosome aberration study with []

Exp.	Preparation interval	Test item	Cell numbers	Mitotic indices		Aberrant cells		
		concentration	in %	in %	in %			
		in µg/mL	of control	of control	incl. gaps*	excl. gaps*	with exchanges	
Exposure period 4 hrs without S9 mix								
I	18 hrs	Solvent control ¹	100.0	100.0	3.0	3.0	0.0	
		Positive control ²	n.t.	63.9	16.0	16.0 ^S	8.0	
		156.3	92.3	107.2	1.0	1.0	0.0	
		312.5 ^P	116.2	91.2	3.0	3.0	1.0	
		625.0 ^P	101.1	80.3	2.0	2.0	0.0	
Exposure period 18 hrs without S9 mix								
II	18 hrs	Solvent control ¹	100.0	100.0	2.5	1.5	0.0	
		Positive control ³	n.t.	76.4	15.0	15.0 ^S	6.5	
		78.1	113.1	75.5	3.0	2.5	1.5	
		156.3 ^P	100.8	67.8	0.0	0.0	0.0	
		312.5 ^P	101.1	58.4	3.5	3.5	0.5	
Exposure period 4 hrs with S9 mix								
I	18 hrs	Solvent control ¹	100.0	100.0	3.5	3.5	0.5	
		Positive control ⁴	n.t.	73.9	13.0	13.0 ^S	8.0	
		156.3	111.3	105.7	1.0	0.5	0.5	
		312.5 ^P	123.2	107.7	2.0	1.5	0.5	
		1250.0 ^P	111.2	80.8	3.5	3.5	1.5	
II	18 hrs	Solvent control ¹	100.0	100.0	1.5	1.5	1.0	
		Positive control ⁴	n.t.	73.0	8.0	8.0 ^S	2.0	
		19.5	72.5	93.7	1.0	1.0	0.5	
		39.1 ^P	87.8	97.5	1.5	1.0	0.0	
		78.1 ^P	96.8	117.3	0.5	0.5	0.0	
		1250.0 ^P	80.8	112.2	1.5	1.5	0.5	

* Inclusive cells carrying exchanges

n.t. Not tested

^P Precipitation occurred at the end of treatment^S Aberration frequency statistically significant higher than corresponding control values¹ DMSO 0.5 % (v/v)² EMS 1000.0 µg/mL³ EMS 500.0 µg/mL⁴ CPA 1.4 µg/mL

12.2 Experiments I and II: Determination of Toxicity

The toxicity of the test item was examined using the determination of the cell number. Cell numbers of two cultures (10 coordinate defined fields per culture) were determined for each experimental group, except the positive control.

Table 2: Number of cells in % of solvent control

Without S9 mix							
Experiment I: 4 hrs exposure				Experiment II: continuous exposure			
Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control	Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control
18 hrs	Solvent control	428	100.0	18 hrs	Solvent control	553	100.0
"	19.5	507	118.3	"	19.5	599	108.3
"	39.1	412	96.1	"	39.1	489	88.4
"	78.1	420	98.0	"	78.1	626	113.1
"	156.3	395	92.3	"	156.3^P	558	100.8
"	312.5^P	498	116.2	"	312.5^P	559	101.1
"	625.0^P	433	101.1	"	625.0 ^P	494	89.2
"	1250.0 ^P	363	84.7	"	1250.0 ^P	n.e.	n.e.
"	2500.0 ^P	337	78.7	"	2500.0 ^P	n.e.	n.e.
"	5000.0 ^P	348	81.2	"	5000.0 ^P	n.e.	n.e.
With S9 mix							
Experiment I: 4 hrs exposure				Experiment II: 4 hrs exposure			
Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control	Preparation interval	Concentration in µg/mL	Number of cells	Cells in % of solvent control
18 hrs	Solvent control	495	100.0	18 hrs	Solvent control	647	100.0
"	19.5	464	93.7	"	4.9	492	76.0
"	39.1	566	114.4	"	9.8	519	80.2
"	78.1	584	118.1	"	19.5	469	72.5
"	156.3	551	111.3	"	39.1^P	568	87.8
"	312.5^P	609	123.2	"	78.1^P	626	96.8
"	625.0 ^P	565	114.2	"	156.3 ^P	386	59.6
"	1250.0^P	550	111.2	"	312.5 ^P	712	110.0
"	2500.0 ^P	373	75.3	"	625.0 ^P	573	88.6
"	5000.0 ^P	429	86.7	"	1250.0^P	523	80.8

Experimental groups evaluated for cytogenetic damage are shown in bold characters

^P Precipitation occurred at the end of treatment

n.e. Not evaluable

12.3 Experiment I

Table 3: Mitotic index;

preparation interval 18 hrs without S9 mix: exposure period 4 hrs;

preparation interval 18 hrs with S9 mix: exposure period 4 hrs

Treatment group	Conc. per mL	S9 mix	Mitotic indices*		Mean	%**
			Absolute 1	Absolute 2		
Solv. control [#]	0.5 %	-	12.5	12.4	12.5	100.0
Pos. control ^{##}	1000.0 µg	-	8.3	7.6	8.0	63.9
Test item	19.5 µg	-	14.9	13.9	14.4	115.7
	39.1 µg	-	12.8	13.2	13.0	104.4
	78.1 µg	-	14.9	16.2	15.6	124.9
"	156.3 µg	-	15.5	11.2	13.4	107.2
"	312.5 µg	-	9.8	12.9	11.4	91.2
"	625.0 µg	-	10.8	9.2	10.0	80.3
"	1250.0 µg	-	9.8	12.6	11.2	90.0
"	2500.0 µg	-	9.6	8.4	9.0	72.3
"	5000.0 µg	-	8.5	5.7	7.1	57.0
Solv. control [#]	0.5 %	+	12.6	13.5	13.1	100.0
Pos. control ^{###}	1.4 µg	+	9.8	9.5	9.7	73.9
Test item	19.5 µg	+	14.4	16.4	15.4	118.0
"	39.1 µg	+	15.3	16.1	15.7	120.3
"	78.1 µg	+	15.6	15.0	15.3	117.2
"	156.3 µg	+	14.5	13.1	13.8	105.7
"	312.5 µg	+	14.6	13.5	14.1	107.7
"	625.0 µg	+	12.1	13.3	12.7	97.3
"	1250.0 µg	+	9.3	11.8	10.6	80.8
"	2500.0 µg	+	9.1	6.3	7.7	59.0
"	5000.0 µg	+	9.2	6.3	7.8	59.4

* The mitotic index was determined in a sample of 1000 cells per culture of each test group in %

** For the positive control groups and the test item groups, the relative values of the mitotic index are related to the solvent controls

DMSO

EMS

CPA

Table 4: Structural chromosome aberrations Experiment I;
preparation interval 18 hrs without S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps*	excl. gaps*	with ex-changes	Gaps		Chromatid type				Chromosome type				Other	
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
					Without S9 mix											
Solvent control: DMSO 0.5 %																
1	100				0	0	3	0	0	0	1	0	0	0	0	0
2	100				0	0	3	0	0	0	1	0	0	0	0	0
1 + 2	200	3.0	3.0	0.0	0	0	6	0	0	0	2	0	0	0	0	0
Positive control: EMS 1000.0 µg / mL																
1	100				0	0	6	1	0	7	1	0	0	0	0	0
2	100				0	0	9	0	0	9	2	0	0	0	0	0
1 + 2	200	16.0	16.0	8.0	0	0	15	1	0	16	3	0	0	0	0	0
Test item: 156.3 µg / mL																
1	100				0	0	1	0	0	0	2	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	1	0	0	0	2	0	0	0	0	0
Test item: 312.5 µg / mL																
1	100				0	0	2	0	0	0	0	0	0	1	0	0
2	100				0	0	1	0	0	1	0	1	0	0	0	0
1 + 2	200	3.0	3.0	1.0	0	0	3	0	0	1	0	1	0	1	0	0
Test item: 625.0 µg / mL																
1	100				0	0	2	0	0	0	2	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	2.0	0.0	0	0	2	0	0	0	2	0	0	0	0	0

* Inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 5: Structural chromosome aberrations Experiment I;
preparation interval 18 hrs with S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations												
		incl. gaps*	excl. gaps*	with ex-changes	Gaps		Chromatid type				Chromosome type				Other		
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd	
					With S9 mix												
Solvent control: DMSO 0.5 %					0	0	1	0	0	0	1	0	0	0	0	0	0
1	100				0	0	7	0	0	2	0	0	0	0	0	0	
2	100				0	0	8	0	0	2	1	0	0	0	0	0	
1 + 2	200	3.5	3.5	0.5	0	0											
Positive control: CPA 1.4 µg / mL					0	0	2	1	0	11	0	0	0	0	0	0	0
1	100				0	0	4	2	0	7	1	0	0	0	0	0	
2	100				0	0	6	3	0	18	1	0	0	0	0	0	
1 + 2	200	13.0	13.0	8.0	0	0											
Test item: 156.3 µg / mL					0	0	0	0	0	1	0	0	0	0	0	0	0
1	100				1	0	0	0	0	0	0	0	0	0	0	0	
2	100				1	0	0	0	0	1	0	0	0	0	0	0	
1 + 2	200	1.0	0.5	0.5													
Test item: 312.5 µg / mL					1	0	0	0	0	0	0	0	0	0	0	0	0
1	100				0	0	2	0	0	2	0	0	0	0	0	0	
2	100				1	0	2	0	0	2	0	0	0	0	0	0	
1 + 2	200	2.0	1.5	0.5													
Test item: 1250.0 µg / mL					0	0	2	0	0	0	0	0	0	0	0	0	0
1	100				0	0	2	0	0	3	0	0	0	0	0	0	
2	100				0	0	4	0	0	3	0	0	0	0	0	0	
1 + 2	200	3.5	3.5	1.5													

* Inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

12.4 Experiment II

Table 6: Mitotic index;

preparation interval 18 hrs without S9 mix: exposure period 18 hrs;

preparation interval 18 hrs with S9 mix: exposure period 4 hrs

Treatment group	Conc. per mL	S9 mix	Mitotic indices*		Mean	%**
			Absolute 1	Absolute 2		
Solv. control [#]	0.5 %	-	11.2	12.1	11.7	100.0
Pos. control ^{##}	500.0 µg	-	8.7	9.1	8.9	76.4
Test item	19.5 µg	-	9.1	10.1	9.6	82.4
"	39.1 µg	-	8.8	9.7	9.3	79.4
"	78.1 µg	-	9.5	8.1	8.8	75.5
"	156.3 µg	-	8.5	7.3	7.9	67.8
"	312.5 µg	-	5.9	7.7	6.8	58.4
"	625.0 µg	-	n.e.	n.e.	n.e.	n.e.
"	1250.0 µg	-	n.e.	n.e.	n.e.	n.e.
"	2500.0 µg	-	n.e.	n.e.	n.e.	n.e.
"	5000.0 µg	-	n.e.	n.e.	n.e.	n.e.
Solv. control [#]	0.5 %	+	14.1	9.6	11.9	100.0
Pos. control ^{###}	1.4 µg	+	7.7	9.6	8.7	73.0
Test item	4.9 µg	+	13.5	11.9	12.7	107.2
"	9.8 µg	+	10.6	12.9	11.8	99.2
"	19.5 µg	+	10.0	12.2	11.1	93.7
"	39.1 µg	+	10.7	12.4	11.6	97.5
"	78.1 µg	+	13.6	14.2	13.9	117.3
"	156.3 µg	+	11.1	9.5	10.3	86.9
"	312.5 µg	+	12.1	13.8	13.0	109.3
"	625.0 µg	+	13.0	14.9	14.0	117.7
"	1250.0 µg	+	13.9	12.7	13.3	112.2

* The mitotic index was determined in a sample of 1000 cells per culture of each test group in %

** For the positive control groups and the test item groups, the relative values of the mitotic index are related to the solvent controls

DMSO

EMS

CPA

n.e. Not evaluable

Table 7: Structural chromosome aberrations Experiment II;
preparation interval 18 hrs without S9 mix: exposure period 18 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps*	excl. gaps*	with ex-changes	Gaps		Chromatid type				Chromosome type				Other	
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
					Without S9 mix											
Solvent control: DMSO 0.5 %																
1	100				2	0	1	0	0	0	1	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	2.5	1.5	0.0	2	0	2	0	0	0	1	0	0	0	0	0
Positive control: EMS 500.0 µg / mL																
1	100				0	0	10	0	0	8	0	1	0	0	0	0
2	100				1	0	5	0	0	7	4	0	0	0	0	0
1 + 2	200	15.0	15.0	6.5	1	0	15	0	0	15	4	1	0	0	0	0
Test item: 78.1 µg / mL																
1	100				1	0	2	0	0	3	0	0	0	0	0	0
2	100				1	0	2	0	0	1	0	0	0	0	0	0
1 + 2	200	3.0	2.5	1.5	2	0	4	0	0	4	0	0	0	0	0	0
Test item: 156.3 µg / mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0
Test item: 312.5 µg / mL																
1	100				0	0	4	0	0	0	0	1	0	0	0	0
2	100				0	0	2	0	0	1	0	0	0	0	0	0
1 + 2	200	3.5	3.5	0.5	0	0	6	0	0	1	0	1	0	0	0	0

* Inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table 8: Structural chromosome aberrations Experiment II;
preparation interval 18 hrs with S9 mix: exposure period 4 hrs

Slide no.	Cells scored	% Aberrant cells			Aberrations											
		incl. gaps*	excl. gaps*	with ex-changes	Gaps		Chromatid type				Chromosome type				Other	
					g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
With S9 mix																
Solvent control: DMSO 0.5 %																
1	100				0	0	1	0	0	1	0	0	0	0	0	0
2	100				0	0	0	0	0	1	0	0	0	0	0	0
1 + 2	200	1.5	1.5	1.0	0	0	1	0	0	2	0	0	0	0	0	0
Positive control: CPA 1.4 µg/mL																
1	100				0	0	5	0	0	3	0	0	0	0	0	0
2	100				0	0	6	1	0	1	0	1	0	0	0	0
1 + 2	200	8.0	8.0	2.0	0	0	11	1	0	4	0	1	0	0	0	0
Test item: 19.5 µg/mL																
1	100				0	0	1	0	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	1	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.5	0	0	1	0	0	1	0	0	0	0	0	0
Test item: 39.1 µg/mL																
1	100				0	1	1	0	0	0	0	0	0	0	0	0
2	100				0	0	1	1	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.0	0	1	2	1	0	0	0	0	0	0	0	0
Test item: 78.1 µg/mL																
1	100				0	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	0	1	0	0	0	0	0	0	0	0
Test item: 1250.0 µg/mL																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	2	0	0	1	0	0	0	0	0	0
1 + 2	200	1.5	1.5	0.5	0	0	2	0	0	1	0	0	0	0	0	0

* Inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap (gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible), b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

12.5 Biometry

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the Fisher's exact test. Evaluation was performed only for cells carrying aberrations excluding gaps.

Table 9: Biometry of Experiment I

	Test group versus solvent control	Preparation interval	Exposure period	S9 mix	p-value
Test group	156.3 µg/mL	18 hrs	4 hrs	-	n.c.
"	312.5 µg/mL	18 hrs	4 hrs	-	n.c.
"	625.0 µg/mL	18 hrs	4 hrs	-	n.c.
"	156.3 µg/mL	18 hrs	4 hrs	+	n.c.
"	312.5 µg/mL	18 hrs	4 hrs	+	n.c.
"	1250.0 µg/mL	18 hrs	4 hrs	+	n.c.
Positive control versus solvent control					
EMS	1000.0 µg/mL	18 hrs	4 hrs	-	$< 0.001^s$
CPA	1.4 µg/mL	18 hrs	4 hrs	+	$< 0.001^s$

n.c. Not calculated as the aberration rate is equal or lower than the corresponding control rate

^s Aberration rate is statistically significant higher than the control rate

Table 10: Biometry of Experiment II

	Test group versus solvent control	Preparation interval	Exposure period	S9 mix	p-value
Test group	78.1 µg/mL	18 hrs	18 hrs	-	0.252
"	156.3 µg/mL	18 hrs	18 hrs	-	n.c.
"	312.5 µg/mL	18 hrs	18 hrs	-	0.111
"	19.5 µg/mL	18 hrs	4 hrs	+	n.c.
"	39.1 µg/mL	18 hrs	4 hrs	+	n.c.
"	78.1 µg/mL	18 hrs	4 hrs	+	n.c.
"	1250.0 µg/mL	18 hrs	4 hrs	+	n.c.
Positive control versus solvent control					
EMS	500.0 µg/mL	18 hrs	18 hrs	-	$< 0.001^s$
CPA	1.4 µg/mL	18 hrs	4 hrs	+	0.001^s

n.c. Not calculated as the aberration rate is equal or lower than the corresponding control rate

^s Aberration rate is statistically significant higher than the control rate

13 ANNEX II

13.1 Chromosome Aberrations: Classification and Criteria

1. Gaps

Gaps are small areas of the chromosome, which are unstained. The chromatids remain aligned as normal and the gap does not extend along the chromatid for a distance greater than the width of a chromatid. If the gap occurs on one chromatid only it is a chromatid gap (g).

2. Chromatid Breaks

Chromatid breaks (b) vary in appearance. The chromatid may remain aligned but show a gap which is too large to classify as a gap. Alternatively, the chromatid may be broken so that the broken fragment is displaced. In some cases, the fragment is not seen at all. A chromatid fragment (f) should be evaluated if the chromosome of origin cannot be identified. In addition, deletions can occur as a result of a break. The missing terminal end of a chromatid in the assessed metaphase is classified as deletion (d).

3. Chromosome Breaks

Chromosome breaks (ib) are breaks in both chromatids of the chromosome. A fragment with two chromatids is formed and this may be displaced by varying degrees. Breaks are distinguished from gaps by the size of the unstained region. A chromosome break is evaluated if the fragment is associated with a chromosome from which it was probably derived. However, fragments are often seen in isolation and are then evaluated as chromatid fragments (if). In addition, isodeletions can occur as a result of an isobreak. The missing terminal end of a chromosome in the assessed metaphase is classified as isodeletion (id).

4. Exchanges

Exchanges are formed by faulty rejoining of broken chromosomes and may be of the chromosome or chromatid type. Chromatid exchanges (ex) have numerous different forms but are generally not further classified. Where multiple exchanges have occurred each exchange point is counted as one chromatid exchange. Chromosome exchanges (cx) generally appear as either a dicentric or a ring form, either of which can be associated with a fragment, which if possible should be evaluated as part of the exchange.

5. Multiple Aberrations

If many aberrations are present in one metaphase, the exact details may not be evaluable. This is particularly the case when chromosome pulverisation (cd) occurs. If the number of aberrations is greater than 4 then the cell is classified as multiple aberrant (ma).

6. Chromosome Number

If the chromosome (centromere) number is 22 ± 1 then it is classified as a diploid cell and evaluated for aberrations. If less than 22 ± 1 chromosomes are counted then the cell is ignored under the assumption, that some chromosomes may have been lost for technical reasons. If greater than 22 ± 1 chromosomes are evaluated then the count is recorded and the cell classified as an aneuploid cell. If multiple copies of the haploid chromosome number (other than diploid) are evaluated then the count is recorded and the cell classified as polyploid. If the chromosomes are arranged in closely apposed pairs, i.e. 4 chromatids instead of 2, the cell is evaluated as endoreduplicated (e).

14 ANNEX III

14.1 Historical laboratory control data

14.1.1 Percentage of aberrant cells in Chinese hamster V79 cell cultures (January 2006 to January 2008)

Without S9 mix				
Aberrant cells excluding gaps (%)				
Treatment/ preparation interval (hours)	No. of performed studies	Range (Min. – Max.)	Mean	Standard deviation
Negative control				
4/18	103	0.0 – 4.0	1.6	0.9
18/18	72	0.0 – 3.5	1.4	0.8
28/28	70	0.0 – 4.0	1.4	1.0
Positive control				
Ethyl methanesulfonate (EMS)				
4/18 (800 µg/mL)	8	9.0 – 17.0	12.8	2.3
4/18 (900 µg/mL)	53	8.0 – 38.0	13.9	6.0
18/18 (400 µg/mL)	10	7.0 – 34.0	14.0	8.1
18/18 (500 µg/mL)	45	8.5 – 41.0	15.5	6.5
28/28 (400 µg/mL)	11	5.0 – 35.0	12.7	8.1
28/28 (500 µg/mL)	43	8.0 – 51.0	26.3	10.7
With S9 mix				
Aberrant cells excluding gaps (%)				
Treatment/ preparation interval	No. of performed studies	Range	Mean	Standard deviation
Negative control				
4/18	110	0.0 – 4.0	2.0	0.9
4/28	66	0.0 – 4.0	1.6	0.9
Positive control				
Cyclophosphamide (CPA)				
4/18 (1.0 µg/mL)	20	5.0 – 21.0	10.7	4.1
4/18 (1.4 µg/mL)	115	7.5 – 38.0	13.2	4.9
4/28 (1.4 µg/mL)	12	4.5 – 23.5	11.0	6.0
4/28 (2.0 µg/mL)	74	7.5 – 44.0	12.1	6.2

**14.1.2 Percentage of polyploid cells in Chinese hamster V79 cell cultures
(January 2006 to January 2008)**

Negative control				
Polyploid cells (%)				
Treatment/ preparation interval (hours)	No. of performed studies	Range (Min. – Max.)	Mean	Standard deviation
Without S9 mix				
4/18	102	1.0 – 4.6	2.3	0.8
18/18	72	0.0 – 5.2	2.6	0.9
28/28	70	1.0 – 4.4	2.4	0.8
With S9 mix				
4/18	110	0.6 – 4.3	2.3	0.8
4/28	66	0.7 – 5.3	2.5	1.0

REPORT

Study Title

DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE ANALYSIS OF [] IN VEHICLE

Author

Dr. K.A. Oudhoff

Study completion date

26 May 2009

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

**NOTOX Project 490629
NOTOX Substance 190683/B**

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2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

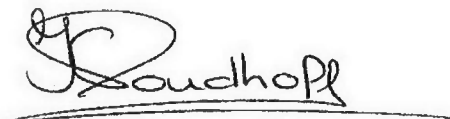
The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by NOTOX.

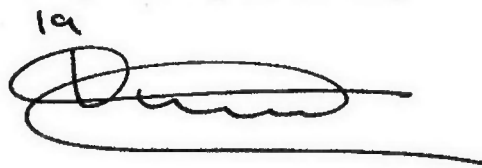
NOTOX B.V.

Dr. K.A. Oudhoff
Study Director



Date: 26 May 2009

Dr. Ir. E. Baltussen
Section Head
Analytical & Physical Chemistry

1a


Dr. Ir. T.H.M. Noij
Head of Chemistry

Date: 26 May 2009



3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.

During the on-site process inspections, procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	06-Mar-09	06-Mar-09	06-Mar-09
	Amendment 1 of protocol	28-Apr-09	28-Apr-09	28-Apr-09
	Report	25-May-09	25-May-09	25-May-09
Process	Analytical and Physical chemistry			
	Test substance handling	21-Apr-09	02-May-09	06-May-09
	Observations/Measurements			

Head of Quality Assurance
C.J. Mitchell B.Sc.

Date: 27 May 09

4. SUMMARY

The analytical method developed for the quantitative analysis of test substance in vehicle was successfully validated for the following parameters:

- Specificity
- Linearity
- Accuracy and repeatability
- Limit of quantification (LOQ)
- Stability of the analytical system and end solutions
- Stability of stock solutions

5. INTRODUCTION

5.1. Preface

Sponsor	Cheil Industries Inc. Mr. Sunghee Ahn 332-2 Gocheon-dong Uiwang-si 437-711, GYEONGGI-DO Korea
Study Monitor	Chemtopia Co., Ltd. Ms. Jinsil Bak No. 1407 Leaders Building 1599-11 Seocho-dong, Seocho-gu 137-876 SEOUL Korea
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Dr. K.A. Oudhoff
Study Plan	Start : 16 March 2009 Completion : 05 May 2009

5.2. Aim of the study

The aim of the study was to develop and to validate an analytical method for the quantitative analysis of the test substance in vehicle.

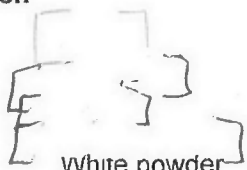
5.3. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens (except specimens requiring refrigeration or freezing) and the final report are retained in the NOTOX archives for a period of at least 2 years after finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. NOTOX will retain information concerning decisions made.

Those specimens requiring refrigeration or freezing will be retained by NOTOX for as long as the quality of the specimens permits evaluation but no longer than three months after finalization of the report.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

6. MATERIALS**6.1. Test substance****6.1.1. Test substance information**

Identification	
Molecular formula	
Molecular weight	
CAS Number	
Description	
Batch	White powder
Purity	20090213
Test substance storage	> 99%
Stability under storage conditions	At room temperature in the dark
Expiry date	Stable
	13 February 2011

6.1.2. Study specific test substance information

There was no study specific test substance information necessary for this study.

6.2. Vehicle

Vehicle Polyethylene glycol 400 (PEG 400)

6.3. Electronic data capture

System control, data acquisition and data processing were performed using the following programme:

- Empower version 5.00 (Waters, Milford, MA, USA)

6.4. List of deviations**6.4.1. List of protocol deviations**

There were no deviations from the protocol.

6.4.2. List of standard operating procedures deviations

Any deviations from standard operating procedures (SOPs) were evaluated and filed in the study file. There were no deviations from SOPs that affected the integrity of the study.

7. VALIDATION OF AN ANALYTICAL METHOD

7.1. Guideline

The study was based on the following guideline:

European Commission: Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, section 4) and Annex III (Part A, section 5) of Directive 91/414, SANCO/3029/99 rev. 4 (11/07/00).

7.2. Reagents

Water	Tap water purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA)
Acetonitrile	Biosolve, Valkenswaard, The Netherlands.
Tetrahydrofuran (THF)	VWR International, Leuven, Belgium
Polyethylene glycol 400 (PEG 400)	Merck, Darmstadt, Germany

All reagents were of analytical grade, unless specified otherwise.

7.3. Performance of the study

A high performance liquid chromatographic method with spectrophotometric detection (HPLC-UV) for the quantitative analysis of the test substance in vehicle was developed. Validation of the analytical method was performed for the following parameters:

Specificity

A test substance solution and blank accuracy sample were analysed by single injection. The analytical method was found to be specific if the blank chromatogram showed no response for the test substance or a response of < 30% of the limit of quantification.

Linearity

Calibration solutions were analysed in duplicate. The response of the calibration solutions was correlated with concentration using regression analysis with a $1/\text{concentration}^2$ weighting factor. A calibration curve with a coefficient of correlation (r) of > 0.99 and back calculated accuracies of the calibration solutions in the range 85-115% was accepted.

Accuracy and repeatability

Accuracy samples were analysed by single injection into the analytical system. The analytical method was considered applicable for the determination of the test substance if the mean accuracy was in the range 85-115% and the coefficient of variation was $\leq 20\%$.

Limit of quantification

The limit of quantification (LOQ) is defined as the lowest concentration level at which an accuracy in the range 85-115% and a repeatability of $\leq 20\%$ is demonstrated. The LOQ was obtained from the data of the accuracy- and repeatability test.

Stability of the analytical system and end solutions

Calibration solutions were injected throughout the validation sequence including the beginning and end. The analytical system and/or end solutions were found to be stable if the coefficient of variation on the responses of the solutions was $\leq 20\%$.

Stability of stock solutions

Stock solutions of the test substance were stored at room temperature for at least 12 hours. Additional calibration solutions were prepared and analysed by single injection. The stock solutions were found to be stable if the coefficient of variation on the response factors of the calibration solutions prepared with fresh- and stored stock solutions was $\leq 10\%$.

7.4. Analytical method

7.4.1. Analytical conditions

Instrument	Alliance Separation Module 2695 (Waters, Milford, MA, USA)
Detector	Dual λ Absorbance Detector 2487 (Waters)
Column	Symmetry Shield RP-18, 100 mm \times 4.6 mm i.d., dp = 3.5 μ m (Waters)
Column temperature	35°C \pm 1°C
Injection volume	10 μ l
Mobile phase	95/5 (v/v) acetonitrile/water
Flow	1 ml/min
UV detection	235 nm

7.4.2. Preparation of solutions

Stock and spiking solutions

Stock and spiking solutions of the test substance were prepared in THF at concentrations of 870 – 2145 mg/l. In order to dissolve the test substance the solutions were ultrasonicated for 10 minutes.

Calibration solutions

Five calibration solutions in the concentration range of 5 – 75 mg/l were prepared from two stock solutions. The end solution of the calibration solutions was 50/50 (v/v) THF/ water.

Accuracy samples

Approximately 500 mg PEG 400 was accurately weighed and spiked with the test substance at a target concentration of 1 or 190 mg/g. The samples were prepared in volumetric flasks of 20 or 100 ml. The flasks were filled up to mark with THF. In order to dissolve the test substance the solutions at the high concentration level were ultrasonicated for 15 minutes.

If necessary, the solutions were diluted with THF to obtain concentrations close to the calibrated range. The solutions were then diluted with water to obtain an end solution of 50/50 (v/v) THF/water and analysed.

The blank accuracy sample was prepared and treated similar to the accuracy samples.

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7.5. Formulas

Response (R)

Peak area of the test substance [units]

Response factor (R_f)

$$R_f = \frac{R}{C_N}$$

where:

 C_N = nominal concentration [mg/l]

Calibration curve

$$R = a C_N + b$$

where:

 a = slope [units \times l/mg] b = intercept [units]Analysed concentration (C_A)

$$C_A = \frac{(R - b)}{a} \times \frac{V \times d}{w} \text{ [mg/g]}$$

where:

 w = weight sample [mg] V = volume volumetric flask [ml] d = dilution factor

Accuracy

$$\frac{C_A}{C_N} \times 100\%$$

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7.6. Results

7.6.1. Specificity

Chromatograms of a test substance solution and the blank accuracy sample are shown in Figure 1 and Figure 2, respectively.

The test substance is a mixture of components. The peak area of the component with a retention time of 5.0 minutes was used as response in the calculations.

The chromatogram of the blank sample showed no peaks at the retention times of the test substance. Since no interferences were detected, the specificity requirements were met and the analytical method was found to be specific for the test substance.

7.6.2. Linearity

The calibration line was constructed using all data points. Figure 3 illustrates the calibration curve and Table 1 shows the statistical parameters. There was a linear relationship between response and test substance concentration in the range of 5.00 – 75.1 mg/l (in end solution). Since the coefficient of correlation (r) was > 0.99 and the back calculated accuracies of the data points were in the range 85-115% the calibration line was accepted.

Table 1 Statistical parameters of the calibration curve

Slope	9.69×10^3
Intercept	-1.13×10^3
Weighting factor	$1/\text{concentration}^2$
r	0.998

7.6.3. Accuracy and repeatability

The results of the accuracy samples are given in Table 2. Since the mean accuracy at each concentration level fell in the criterion 85-115% and the coefficient of variation was $\leq 20\%$ the analytical method was accepted for the analysis of the test substance in vehicle in the target concentration range of 1 - 190 mg/g.

Table 2 Accuracy samples

Target concentration [mg/g]	Nominal concentration [mg/g]	Analysed concentration [mg/g]	Accuracy [%]	Mean accuracy [%]	Coefficient of variation [%]
1	0.999	1.05	105	106	1.0
	0.996	1.07	107		
	0.988	1.05	107		
	0.989	1.03	104		
	0.964	1.02	106		
190	183	193	106	104	1.4
	194	199	103		
	186	197	106		
	189	195	103		
	186	194	105		

7.6.4. Limit of quantification

The limit of quantification (LOQ) was assessed at 1 mg/g in vehicle.

7.6.5. Stability of the analytical system and end solutions

The results of the stability of the analytical system and end solutions are given in Table 3. Since the coefficient of variation at both concentration levels was $\leq 20\%$ the analytical system and end solutions were stable over at least a 5.3 hour time interval.

Table 3 Stability of the analytical system and end solutions

Nominal concentration [mg/l]	Elapsed time [hours]	Coefficient of variation [%]
5.00	7.1	0.58 (n=4)
75.1	5.3	0.42 (n=4)

7.6.6. Stability of stock solutions

The results of the stability of the stock solutions are given in Table 4. The stock solutions were analysed at a nominal concentration of 75 mg/l. Since the coefficient of variation on the response factors of the calibration solutions was $\leq 10\%$ the stock solutions were stable when stored at room temperature for at least 8 days.

Table 4 Stability of the stock solutions

Nominal concentration		Elapsed time [days]	Coefficient of variation [%]
Stored [mg/l]	Fresh [mg/l]		
870 2145	1330 1900	8	2.0

7.7. Conclusion

The analytical method was validated for the following parameters:

Specificity	specific
Linearity	$r = 0.998$
Accuracy	104 and 106%
Repeatability	1.0 and 1.4%
Limit of quantification	1 mg/g
Stability analytical system and end solutions	stable
Stability stock solutions	stable

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7.8. Figures

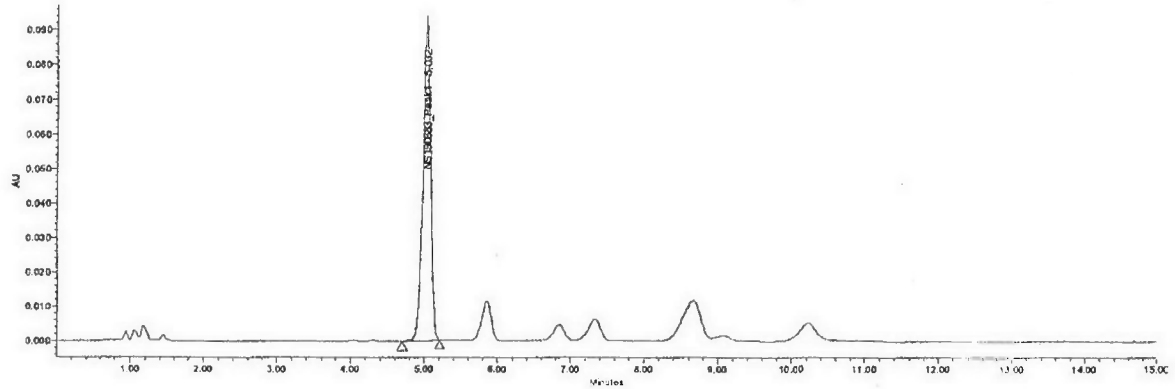


Figure 1 HPLC-UV chromatogram of a 75.1 mg/l test substance solution [res. id. 2512].

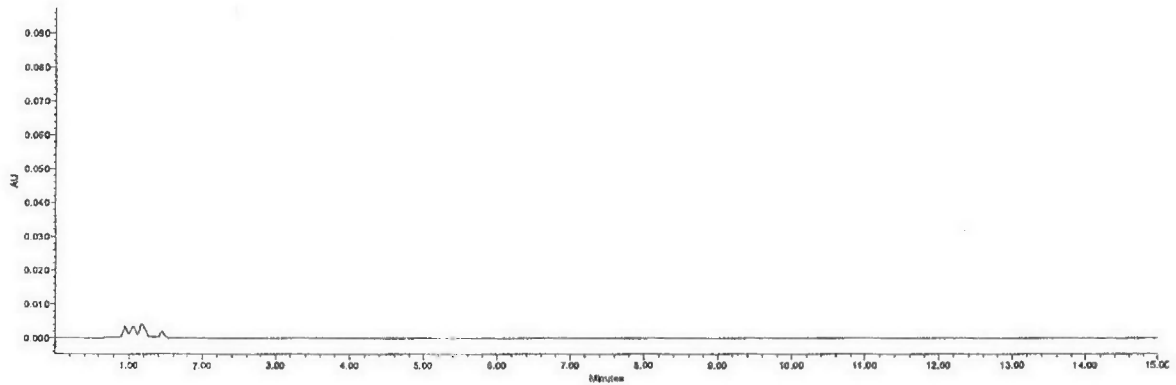


Figure 2 HPLC-UV chromatogram of the blank accuracy sample [res. id. 2514].

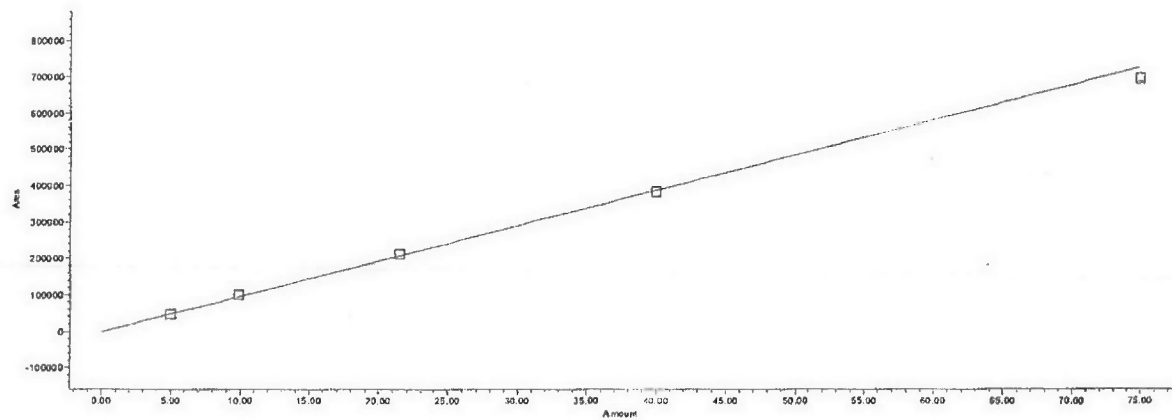


Figure 3 Regression line: response of the test substance as a function of concentration [cal. curve id. 2502].

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Appendix I GLP certificate



voedsel en waren autoriteit

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and
according to Directive 2004/9/EC, the conformity with the OECD Principles of GLP
was assessed on 16 – 20 February, 2009 at

NOTOX B.V.
Hambakenwetering 7
5231 DD 's Hertogenbosch

It is herewith confirmed that the afore-mentioned test facility is currently operating
in compliance with the OECD Principles of Good Laboratory Practice in the
following area of expertise: Physical-chemical testing; Toxicity studies; Mutagenicity
studies; Environmental toxicity studies on aquatic and terrestrial organisms; Studies
on behaviour in water, soil, and air, bioaccumulation; Residue studies; Analytical and
clinical chemistry testing; Kinetic and metabolism studies, Safety Pharmacology.



Den Haag, 30 March 2009

Dr. Th. Helder

Manager GLP Compliance Monitoring Program

Food and Consumer Product Safety Authority (VWA)
Prinses Beatrixlaan 2, 2595 AL Den Haag
Postbus 19506, 2500 CM Den Haag
The Netherlands

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REPORT

Study Title

**REPEATED DOSE 28-DAY ORAL TOXICITY STUDY WITH
[]
BY DAILY GAVAGE
IN THE RAT
FOLLOWED BY A 14-DAY RECOVERY PERIOD**

Author

F.M. van Otterdijk, M.Sc.

Study completion date

03 June 2009

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

**NOTOX Project 490627
NOTOX Substance 190683/B**

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2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by NOTOX.

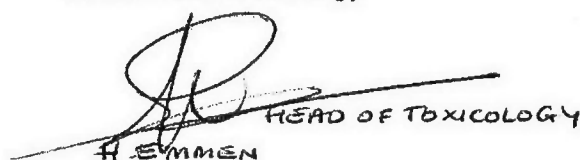
NOTOX B.V.

F.M. van Otterdijk, M.Sc.
Study Director

Drs. M.S. Teunissen
Section Head Toxicology



Date: 03 June 2009



HEAD OF TOXICOLOGY
H. EMMEN

Date: 03 June 2009

3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.

During the on-site process inspections procedures applicable to this type of study were inspected

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	04-Mar-09	04-Mar-09	04-Mar-09
	Amendment 1 of Protocol	10-Mar-09	10-Mar-09	10-Mar-09
	Amendment 2 of Protocol	12-Mar-09	12-Mar-09	12-Mar-09
	Amendment 3 of Protocol	30-Mar-09	30-Mar-09	30-Mar-09
	Amendment 4 of Protocol	31-Mar-09	31-Mar-09	31-Mar-09
	Necropsy including organ weighing	10-Apr-09	10-Apr-09	10-Apr-09
	Amendment 5 of Protocol	04-May-09	04-May-09	04-May-09
	Report	18-May-09	19-May-09	20-May-09
	Amendment 6 of Protocol	28-May-09	28-May-09	28-May-09
Process	Test substance unit Test substance handling	11-Feb-09	16-Feb-09	20-Feb-09
	SPF unit Test substance handling Exposure Observations/Measurements Specimen handling	09-Feb-09	16-Feb-09	18-Feb-09
	Clinical pathology unit Observations/Measurements Specimen handling	03-Mar-09	09-Mar-09	09-Mar-09
	Histology unit Specimen handling	20-Apr-09	25-Apr-09	01-May-09
	Pathology unit Observations/Measurements	17-Mar-09	24-Mar-09	07-Apr-09
	Analytical and physical chemistry Test substance handling Observations/Measurements	20-Apr-09	22-Apr-09	27-Apr-09

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QUALITY ASSURANCE STATEMENT (Continued)

Head of Quality Assurance
C.J. Mitchell B.Sc.



Date: 3-June-09

4. SUMMARY

Title

Repeated dose 28-day oral toxicity study with [] by daily gavage in the rat, followed by a 14-day recovery period.

Guidelines

The study was based on the following guidelines.

- EC No 440/2008, B.7 Repeated Dose (28 days) Toxicity (oral), 2008.
- OECD 407, Repeated Dose 28-day Oral Toxicity Study in Rodents, 2008.
- Japanese Chemical Substances Control Law 1987, Notification of Nov. 21 2003 by MHLW (No. 1121002), METI (No. 2) and ME (No. 031121002).
- OPPTS 870.3050, Repeated dose 28-day oral toxicity study in rodents. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-00-366, 2000.

Rationale for dose levels

Based on the results of a 5-day range finding study (NOTOX Project 490628), the dose levels for this 28-day oral gavage study were selected to be 0, 30, 300 and 1000 mg/kg/day.

Study outline

The test substance, formulated in vehicle, was administered daily for 28 days by oral gavage to SPF-bred Wistar rats. One control group and three treated groups were tested, each consisting of 5 males and 5 females. An extra 5 animals per sex in the control and high dose group were allowed 14 days of recovery.

Evaluated parameters

Chemical analyses of formulations were conducted during the study to assess accuracy, homogeneity and stability over a maximum of 6 hours.

The following parameters were evaluated: clinical signs daily; functional observation tests in week 4; body weight and food consumption weekly; clinical pathology and macroscopy at termination; organ weights and histopathology on a selection of tissues.

Results

Formulation analyses confirmed that formulations of test substance in polyethylene glycol 400 were prepared accurately and homogeneously, and were stable over at least 6 hours.

No toxicologically significant changes were noted in any of the observed/measured parameters.

Conclusion

From the results presented in this report a No Observed Adverse Effect Level (NOAEL) for [] of 1000 mg/kg/day was established. A No Observed Effect Level (NOEL) of 30 mg/kg/day was established.

5. INTRODUCTION

5.1. Preface

Information concerning the dose range finding study is given in Appendix 3 (NOTOX project 490628)

Sponsor	Cheil Industries Inc. Mr. Sunghee Ahn 332-2 Gocheon-dong Uiwang-si 437-711, GYEONGGI-DO Korea
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Test facility Management	H.H. Emmen, M.Sc. (NOTOX B.V.): <u>harry.emmen@notox.nl</u>
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands

5.2. Study plan

Experimental starting date	05 March 2009 (allocation pilot study)
Treatment	13 March 2009 to 09 April 2009
Start Recovery	10 April 2009
Blood sampling + urine collection	10 April 2009 (all groups) 24 April 2009 (Recovery groups)
Necropsy	10 April 2009 (Main groups) 24 April 2009 (Recovery groups)
Experimental completion date	24 April 2009 (end of in-life phase)

5.3. Aim of the study

The nature and purpose of this toxicity study was to assess the toxic potential of the test substance when administered to rats by daily oral gavage for a period of 28 days, followed by a 14-day recovery period. A No Observed Adverse Effect Level (NOAEL) was evaluated.

This study should provide a rational basis for toxicological risk assessment in man. The oral route was selected as it is a possible route of human exposure during manufacture, handling or use of the test substance.

5.4. Guidelines

As required by the Dutch Act on Animal Experimentation (February 1997), the protocol was reviewed and agreed by the Animal Welfare Officer and the Ethical Committee of NOTOX (DEC NOTOX 97-03-15). The study procedures described in this report were based on the following guidelines:

1. Commission regulation (EC) No 440/2008 Part B: Methods for the Determination of Toxicity and other Health Effects; B.7: "Repeated Dose (28 days) Toxicity (oral)". Official Journal of the European Communities No. L142, May 2008.
2. Organization for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No. 407: "Repeated Dose 28-day Oral Toxicity Study in Rodents", Paris Cedex, 03 October 2008.
3. Japanese Chemical Substances Control Law 1987 according to the notification of November 21, 2003 by Ministry of Health, Labor and Welfare (No. 1121002), Ministry of Economy, Trade and Industry (No. 2) and Ministry of Environment (No. 031121002).
4. United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.3050, Repeated dose 28-day oral toxicity study in rodents. Office of Prevention, Pesticides and Toxic Substances (7101), EPA 712-C-00-366, July 2000.

5.5. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens (except specimens requiring refrigeration or freezing) and the final report are retained in the NOTOX archives for a period of at least 2 years after finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. NOTOX will retain information concerning decisions made.

Those specimens requiring refrigeration or freezing will be retained by NOTOX for as long as the quality of the specimens permits evaluation but no longer than three months after finalization of the report.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

6. MATERIALS AND METHODS

6.1. Test substance

6.1.1. Test substance information

Identification	
Molecular formula	
Molecular weight	
CAS Number	
Description	White powder
Batch	20090213
Purity	> 99%
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	13 February 2011

6.1.2. Study specific test substance information

pH	Not indicated
Solubility in polyethylene glycol	Not indicated

6.1.3. Test substance Formulation

Vehicle	Polyethylene glycol 400 (Merck, Darmstadt, Germany), specific gravity 1.125.
Rationale for vehicle	Based on trial formulations performed at NOTOX and on information provided by the Sponsor.
Method of formulation	Formulations (w/w) were prepared daily within 6 hours prior to dosing and were homogenized to a visually acceptable level. Adjustment was made for specific gravity of the vehicle. No correction was made for the purity of the test substance.
Storage conditions	At ambient temperature.

6.1.4. Chemical analysis of dose preparations

The analytical method used was validated under NOTOX project 490629.

Initially, samples of formulations were analyzed on day 12 for homogeneity (highest and lowest concentration) and accuracy of preparation (all concentrations). Stability in vehicle over 6 hours was also determined (highest and lowest concentration). Additional analyses were conducted on formulations prepared on day 15 since homogeneity and accuracy results of the initial analyses were below the acceptable range. Stability was determined over a 4-hour period instead of a 6-hour period.

After the in-life phase, additional analyses were conducted since the initial analytical method validation experiments, and the preparation of the calibration solutions and procedural recovery samples for formulation analyses on day 12 and 15 were inadvertently conducted with NOTOX test substance 190683/A instead of 190683/B. Therefore, the method validation experiments were repeated using NOTOX test substance 190683/B. As a consequence, formulation analyses (with preparation of the calibration solutions and procedural recovery samples) were repeated using the revalidated analytical method with NOTOX test substance 190683/B. Homogeneity and stability in vehicle over 6 hours (highest and lowest concentration), and accuracy of preparation (all concentrations) was determined.

Only the analytical results obtained with the revalidated method are reported.

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The accuracy of preparation was considered acceptable if the mean measured concentrations were 85-115%. Homogeneity was demonstrated if the coefficient of variation was $\leq 10\%$. Formulations were considered stable if the relative difference before and after storage was maximally 10%.

6.2. Test system

Test System	Rat: CrI:WI(Han) (outbred, SPF-Quality).
Rationale	Recognized by international guidelines as the recommended test system (e.g. EPA, FDA, OECD and EC).
Source	Charles River Deutschland, Sulzfeld, Germany.
Total number of animals	30 males, 30 females. (females were nulliparous and non-pregnant).
Age at start of treatment	Approximately 7 weeks.
Identification	Earmark and tattoo.
Randomization	By computer-generated random algorithm according to body weight, with all animals within $\pm 20\%$ of the sex mean.
Acclimatization period	At least 5 days before the start of treatment under laboratory conditions.
Health inspection	Prior to commencement of treatment to ensure that the animals were in a good state of health.

6.3. Allocation

Group	Dose level ¹ mg/kg/day	Dose volume mL/kg	Number of animals		Animal numbers	
			Males	Females	Males	Females
1 Main	0 (vehicle)	5	5	5	1-5	31-35
1 Recovery	0 (vehicle)	5	5	5	6-10	36-40
2 Main	30	5	5	5	11-15	41-45
3 Main	300	5	5	5	16-20	46-50
4 Main	1000	5	5	5	21-25	51-55
4 Recovery	1000	5	5	5	26-30	56-60

¹ The dose levels were selected on the basis of a 5-day dose range finding study (NOTOX Project 490628). A summary of the results is included in this report (see Appendix 3).

6.4. Animal husbandry

Room number 7.

Conditions Animals were housed in a controlled environment, in which optimal conditions were considered to be approximately 15 air changes per hour, a temperature of $21.0 \pm 3.0^\circ\text{C}$ (actual range: $18.8 - 21.1^\circ\text{C}$), a relative humidity of 30-70% (actual range: 30 - 92%) and 12 hours artificial fluorescent light and 12 hours darkness per day. Temporary fluctuations from the light/dark cycle (with a maximum of 1 hour) occurred due to performance of functional observations in the room. Based on laboratory historical data, these fluctuations were considered not to have affected the study integrity.

Accommodation Group housing of 5 animals per sex in Macrolon cages (MIV type, height 18 cm; during overnight activity monitoring individual housing in MIII type; height 15 cm.) with sterilized sawdust as bedding material (Litalabo, S.P.P.S., Argenteuil, France) and paper as cage-enrichment (Enviro-dri, Wm. Lilico & Son (Wonham Mill Ltd), Surrey, United Kingdom).

No cage-enrichment was provided during overnight activity monitoring.
Certificates of analysis were examined and then retained in the NOTOX archives.

Diet	Free access to pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany). Results of analyses for nutrients and contaminants were examined and archived. A sample (approximately 500 grams) of each batch was retained at room temperature until finalization of the study report.
Water	Free access to tap water. Certificates of analysis (performed quarterly) were examined and archived.

Analysis of bedding, paper, diet and water did not reveal any findings that were considered to have affected study integrity.

6.5. Treatment

Method	Oral gavage, using a plastic feeding tube. Formulations were placed on a magnetic stirrer during dosing.
Frequency	Once daily, 7 days per week, approximately the same time each day with a maximum of 6 hours difference between the earliest and latest dose.
Dose volume	5 mL/kg body weight. Actual dose volumes were calculated weekly according to the latest body weight.
Duration of treatment	28 days. Main animals were dosed up to the day prior to necropsy, and Recovery animals were dosed up to the day prior to start of the recovery period.
Duration of recovery	14 days.

6.6. Observations

Mortality / Viability	At least twice daily.
Clinical signs	At least once daily, detailed clinical observations were made in all animals. Once prior to start of treatment and at weekly intervals, this was also performed outside the home cage in a standard arena. The time of onset, degree and duration were recorded. All symptoms were recorded and graded according to fixed scales: Maximum grade 1: grade 0 = absent, grade 1 = present Maximum grade 3 or 4: grade 1 = slight, grade 2 = moderate, grade 3 = severe, grade 4 = very severe

Functional Observations

During week 4 of treatment, the following tests were performed on all animals: (abbreviations mentioned in the respective tables indicated between brackets):

- hearing ability (HEARING), pupillary reflex (PUPIL L/R), static righting reflex (STATIC R) and grip strength (GRIP) (Score 0 = normal/present, score 1 = abnormal/absent).
- motor activity test (recording period: 12 hours during overnight for individual animals, using a computerized monitoring system, Pearson Technical Services, Debenham, Stowmarket, England).

Since the above mentioned measurements did not reveal treatment-related effects, the functional observation tests were not performed at the end of the recovery phase.

Body weights

Weekly.

Food consumption

Weekly.

Water consumption

Subjective appraisal was maintained during the study, but no quantitative investigation introduced as no effect was suspected.

6.7. Clinical laboratory investigations

Blood samples were collected from all animals under iso-flurane anaesthesia (Abbott Laboratories Ltd., Hoofddorp, The Netherlands) immediately prior to scheduled *post mortem* examination at the end of the treatment and recovery phase, between 7.00 and 11.30 a.m.. Animals were deprived of food overnight (for a maximum of 20 hours), but water was available. Blood samples were drawn from the retro-orbital sinus and collected into tubes (Greiner Bio-One, Bad Haller, Austria) prepared with EDTA for haematological parameters (0.5 mL), with citrate for clotting tests (0.45 mL) and Li-heparin treated tubes for clinical biochemistry parameters (0.5 mL). An additional blood sample (0.25 mL) was collected into untreated tubes for determination of bile acids. The following parameters were determined:

Parameter	Abbreviation	Unit
Haematology^a		
White blood cells	WBC	10 ⁹ /L
Differential leucocyte count neutrophils, lymphocytes, monocytes, eosinophils, basophils		%WBC
Red blood cells		10 ¹² /L
Reticulocytes		%RBC
Red blood cell distribution width	RDW	%
Haemoglobin		mmol/L
Haematocrit		L/L
Mean corpuscular volume	MCV	fL
Mean corpuscular haemoglobin	MCH	fmol
Mean corpuscular haemoglobin concentration	MCHC	mmol/L
Platelets		10 ⁹ /L
Clotting Potential^b		
Prothrombin time	PT	s
Activated Partial thromboplastin time	APTT	s
Clinical Biochemistry^c		
Alanine aminotransferase	ALAT	U/L
Aspartate aminotransferase	ASAT	U/L
Alkaline phosphatase	ALP	U/L
Total Protein		g/L
Albumin		g/L
Total Bilirubin		µmol/L
Urea		mmol/L
Creatinine		µmol/L
Glucose		mmol/L
Cholesterol		mmol/L
Sodium		mmol/L
Potassium		mmol/L
Chloride		mmol/L
Calcium		mmol/L
Inorganic Phosphate	Inorg. Phos	mmol/L
Bile acids		mmol/L

^a Instrumentation: ADVIA 120 (Siemens Medical Solutions Diagnostics).

^b Instrumentation: STA Compact (Roche Diagnostics).

^c Instrumentation: Olympus AU400 (Goffin Meyvis).

6.8. Pathology**6.8.1. Necropsy**

All animals were deeply anaesthetized using iso-flurane vapor (Abbott Laboratories Ltd., Hoofddorp, The Netherlands) and subsequently exsanguinated. All animals assigned to the study were necropsied and descriptions of all macroscopic abnormalities recorded. Samples of the following tissues and organs were collected from all animals at necropsy and fixed in 10% buffered formalin (neutral phosphate buffered 4% formaldehyde solution, Klinipath, Duiven, The Netherlands):

Identification marks: not processed	Ovaries
Adrenal glands	(Pancreas)
(Aorta)	Peyer's patches [jejunum, ileum] if detectable
Brain [cerebellum, mid-brain, cortex]	Pituitary gland
Caecum	(Preputial gland)
Cervix	Prostate gland
(Clitoral gland)	Rectum
Colon	(Salivary glands - mandibular, sublingual)
Duodenum	Sciatic nerve
Epididymides *	Seminal vesicles including coagulating gland
Eyes (including optic nerve and hardierian gland) *	Skeletal muscle
(Female mammary gland area)	(Skin)
Femur including joint	Spinal cord -cervical, midthoracic, lumbar
Heart	Spleen
Ileum	Sternum with bone marrow
Jejunum	Stomach
Kidneys	Testes *
(Larynx)	Thymus
(Lacrimal gland, exorbital)	Thyroid including parathyroid [if detectable]
Liver	(Tongue)
Lung, infused with formalin	Trachea
Lymph nodes - mandibular, mesenteric	Urinary bladder
(Nasopharynx)	Uterus
(Oesophagus)	Vagina
	All gross lesions

* Fixed in modified Davidson's solution, prepared at NOTOX using Formaldehyde 37-40%, Ethanol, Acetic acid - glacial (all Merck, Darmstadt, Germany) and Milli-Ro water (Millipore Corporation, Bedford, USA). Tissues were transferred to formalin after fixation for at least 24 hours.

Tissues/organs mentioned in parentheses were not examined by the pathologist, since no signs of toxicity were noted at macroscopic examination.

6.8.2. Organ weights

The following organ weights and terminal body weight were recorded on the scheduled day of necropsy:

Adrenal glands	Spieen
Brain	Testes
Epididymides	Thymus
Heart	Uterus (including cervix)
Kidneys	Prostate ¹
Liver	Seminal vesicles including coagulating glands ¹
Ovaries	Thyroid including parathyroid ¹

¹ weighed when fixed for at least 24 hours.

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6.8.3. Histotechnology

All organ and tissue samples, as defined under Histopathology (following), were processed, embedded, cut at a thickness of 2-4 micrometers and stained with haematoxylin and eosin (Klinipath, Duiven, The Netherlands).

6.8.4. Histopathology

The following slides were examined by a pathologist:

- all tissues collected at the scheduled sacrifice from all Main group 1 and 4 animals,
- all gross lesions.

All abnormalities were described and included in the report. An attempt was made to correlate gross observations with microscopic findings.

6.9. Electronic data capture

Observations/measurements in the study were recorded electronically using the following programme(s):

- REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA): Environmental monitoring.
- TOXDATA version 8.0 (NOTOX B.V., 's-Hertogenbosch, The Netherlands): Mortality / Clinical signs / Body weights / Food consumption / Organ weights.
- MAMS version 6.5 (Pearson Technical Services, Suffolk, Great Britain): Motor activity measurement
- Advia 120 version V.3.1.8.0.MS (Siemens Medical Solutions Diagnostics, Breda, The Netherlands): Haematology
- Sta Compact version 1.06.06 (Stago Instruments, Gennevilliers, France): Clotting parameters
- Olympus AU 400 version 8.2A (Goffin-Meyvis, Etten-Leur, The Netherlands): Clinical laboratory investigations
- Pathdata version 6.2D (Pathology Data Systems, Basel, Switzerland): Histopathology
- Empower version 5.00 (Waters, Milford, MA, USA): Formulation analysis,

6.10. Interpretation

The following statistical methods were used to analyze the data:

- If the variables could be assumed to follow a normal distribution, the Dunnett-test¹ (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex.
- The Steel-test² (many-to-one rank test) was applied when the data could not be assumed to follow a normal distribution.
- The exact Fisher-test³ was applied to frequency data.

All tests were two-sided and in all cases $p < 0.05$ was accepted as the lowest level of significance. Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables. Test statistics were calculated on the basis of exact values for means and pooled variances. Individual values, means and standard deviations may have been rounded off before printing. Therefore, two groups may display the same printed means for a given parameter, yet display different test statistics values.

6.11. List of deviations

6.11.1. List of protocol deviations

- 1 Animal no. 25 was not identified by means of a tattoo (indicating the cage number).
Evaluation: This animal was identified by an earmark (indicating the animal number).
No conditions occurred during this study by which the missing tattoo may have had an adverse effect on the study integrity.
- 2 Temporary deviations from the maximum level of relative humidity occurred.
Evaluation: Laboratory historical data do not indicate an effect of the deviations.
- 3 An arena observation was conducted on day 8 of the recovery period.
Evaluation: Additional information.
- 4 190683/A instead of 190683/B was used during chemical analysis of formulations (i.e. for preparation of the calibration solutions and procedural recovery samples).
Evaluation: The analytical method was revalidated using 190683/B, and formulation analyses (with preparation of calibration solutions and procedural recovery samples) were repeated using the revalidated method.

The study integrity was not adversely affected by the deviations.

6.11.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

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7. RESULTS

7.1. Analysis of dose preparations (see also APPENDIX 4)

The concentrations analysed in the formulations of Group 2, Group 3 and Group 4 were in agreement with target concentrations (i.e. mean accuracies between 85% and 115%).

The formulations of Group 2 and Group 4 were homogeneous (i.e. coefficient of variation $\leq 10\%$).

Analysis of Group 2 and Group 4 formulations after storage yielded a relative difference of $\leq 10\%$. Based on this, the formulations were found to be stable during storage at room temperature for at least 6 hours.

In Group 1 formulations, no test substance was detected.

7.2. Observations

7.2.1. Mortality

No mortality occurred during the study period.

7.2.2. Clinical signs

No clinical signs of toxicity were noted during the observation period.

Salivation as seen after dosing among all dose groups (with the highest incidence at 1000 mg/kg/day) was considered to be a physiological response rather than a sign of systemic toxicity considering the nature and minor severity of the effect and its time of occurrence (i.e. after dosing, during treatment only). Incidental findings that were noted included chromodacryorrhoea, maculate erythema of the right ear, a dark right eye and opacity of the right eye. These findings occurred within the range of background findings to be expected for rats of this age and strain which are housed and treated under the conditions in this study. At the incidence observed, these were considered signs of no toxicological significance.

7.2.3. Functional observations

Hearing ability, pupillary reflex, static righting reflex and grip strength were normal in all animals. The variation in motor activity did not indicate a relation with treatment.

The statistically significant lower motor activity counts of males at 30 and 300 mg/kg/day as recorded by the high sensors occurred in the absence of a dose related trend. Therefore, these changes were considered to be without toxicological relevance.

7.2.4. Body weights

No toxicologically significant changes in body weights and body weight gain were noted.

Any statistically significant variations in body weights or body weight gain were of a slight nature and remained within the range considered normal for rats of this age and strain. These variations included lower body weights for females at 1000 mg/kg/day at the end of treatment, and lower body weight gain for females at 300 and 1000 mg/kg/day at the end of treatment and for females at 1000 mg/kg/day also on day 1 and 14 of the recovery period.

7.2.5. Food consumption

Food consumption before or after allowance for body weight was similar between treated and control animals.

7.3. Clinical laboratory investigations

7.3.1. Haematology

No toxicologically relevant changes occurred in haematological parameters of treated rats.

Any statistically significant changes at the end of the treatment period were considered to be of no toxicological significance as they occurred in the absence of a treatment-related distribution and/or remained within the range considered normal for rats of this age and strain. These changes included lower haemoglobin levels in males at 30 and 1000 mg/kg/day, lower relative eosinophil counts in females at 30, 300 and 1000 mg/kg/day, and lower platelet counts in females at 30 and 1000 mg/kg/day. The lower mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in females at 1000 mg/kg/day were only slightly outside the range considered normal for rats of this age and strain, but since these variations were minor and without a dose-related trend, they were considered to be without toxicological significance. Statistically significant changes at the end of the recovery period were also within the range considered normal for rats of this age and strain, and occurred in the absence of similar or more pronounced changes at the end of the treatment period. No toxicological significance was therefore ascribed to these changes, which included higher reticulocyte counts and lower mean corpuscular haemoglobin concentration (MCHC) in males at 1000 mg/kg/day, and higher red cell distribution width (RDW) and lower platelet counts in females at 1000 mg/kg/day.

7.3.2. Clinical biochemistry

Higher cholesterol levels were recorded for females at 300 and 1000 mg/kg/day at the end of the treatment period, and had resolved at the end of the recovery period.

At the end of the recovery period, creatinine and potassium levels were higher in females at 1000 mg/kg/day. These values exceeded the range considered normal for rats of this age and strain.

The lower total bilirubin levels in males at 30, 300 and 1000 mg/kg/day and lower bile acid levels in males at 300 and 1000 mg/kg/day occurred in the absence of a treatment-related distribution and remained within the range considered normal for rats of this age and strain. Also, an opposite effect (i.e. higher levels as compared to controls) would be expected in case of target organ toxicity. These changes were therefore considered not to be related to treatment with the test substance.

The statistically significant higher inorganic phosphate level in males at 1000 mg/kg/day at the end of the recovery period was absent at the end of the treatment period and remained within the range considered normal for rats of this age and strain. This change was therefore considered to be without toxicological significance.

7.4. Pathology

7.4.1. Macroscopic examination

Necropsy did not reveal any toxicologically relevant alterations.

Reduced size of the seminal vesicles, fluid in the uterus, red discolouration of the clitoral glands and tan foci on the clitoral glands occurred within the range of background findings noted in this type of study, and considered to be of no toxicological significance. No macroscopic abnormalities were noted among males at 30, 300 and 1000 mg/kg/day and females at 30 mg/kg/day.

7.4.2. Organ weights

Liver to body weight ratios of males and females at 300 and 1000 mg/kg/day were slightly higher at the end of the treatment period, achieving a level of statistical significance.

Thyroid to body weight ratio was slightly higher for females at 1000 mg/kg/day at the end of the treatment period.

At the end of the recovery period, relative liver and thyroid weights were similar to control levels.

Other statistically significant changes at the end of the treatment period were considered to be of no toxicological significance as these were within the range considered normal for rats of this age and strain and/or occurred without a dose-related trend. These variations included higher relative seminal vesicle weight for males at 1000 mg/kg/day, higher relative testes weight for males at 30 mg/kg/day, and lower thymus weight for females at 300 mg/kg/day. The statistically significant higher brain to body weight ratio of females at 1000 mg/kg/day at the end of the recovery period, was considered to be related to the slightly lower terminal body weight, and hence without toxicological relevance.

7.4.3. Microscopic examination (see also APPENDIX 5)

There were no microscopic findings recorded which could be attributed to treatment with the test substance.

All microscopic findings were within the range of background pathology encountered in Wistar rats of this age and strain and occurred at similar incidences and severity in both control and treated rats.

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8. DISCUSSION AND CONCLUSION

Wistar rats were treated with [] for 28 consecutive days by daily oral gavage at dose levels up to 1000 mg/kg/day, followed by a 14-day treatment-free recovery period.

Treatment with [] was well tolerated; no clinical signs of toxicity were noted, functional observation tests revealed no abnormalities, and body weight and food consumption remained normal.

Analysis of blood revealed higher cholesterol levels for females at 300 and 1000 mg/kg/day at the end of the treatment period, and higher creatinine and potassium levels in females at 1000 mg/kg/day. Although these values exceeded the range considered normal for rats of this age and strain, there were no histopathological correlates. Also, either these changes had recovered during the recovery period (cholesterol) or these were not apparent at the end of the treatment period (creatinine and potassium). For creatinine and potassium a similar or more pronounced change would be expected at the end of the treatment period. No further changes in haematological or clinical biochemistry parameters were noted.

Necropsy at the end of the treatment period showed slightly higher liver to body weight ratios of males and females at 300 and 1000 mg/kg/day. Thyroid to body weight ratio was also slightly higher for females at 1000 mg/kg/day at the end of the treatment period. At the end of the recovery period, relative liver and thyroid weights were similar to control levels. Given that these changes were slight in nature and occurred without histopathological correlates, these were considered to be of no toxicological significance.

No toxicologically significant changes were noted at microscopic examination of organs/tissues collected at scheduled sacrifice.

Since no toxicologically significant changes were noted in any of the observed/measured parameters, a No Observed Adverse Effect Level (NOAEL) for [] of 1000 mg/kg/day was established. A No Observed Effect Level (NOEL) of 30 mg/kg/day was established.

9. REFERENCES

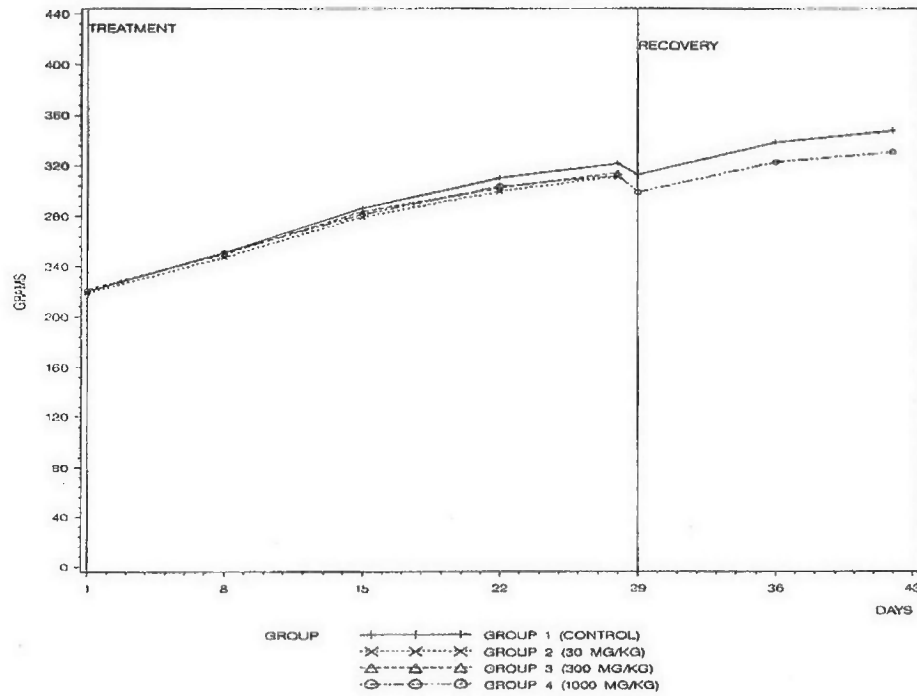
- 1 C.W. Dunnett, A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J. Amer. Stat. Assoc. 50, 1096-1121 (1955).
- 2 R.G. Miller, Simultaneous Statistical Inference, Springer Verlag, New York (1981).
- 3 R.A. Fisher, Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh (1950).

APPENDIX 1

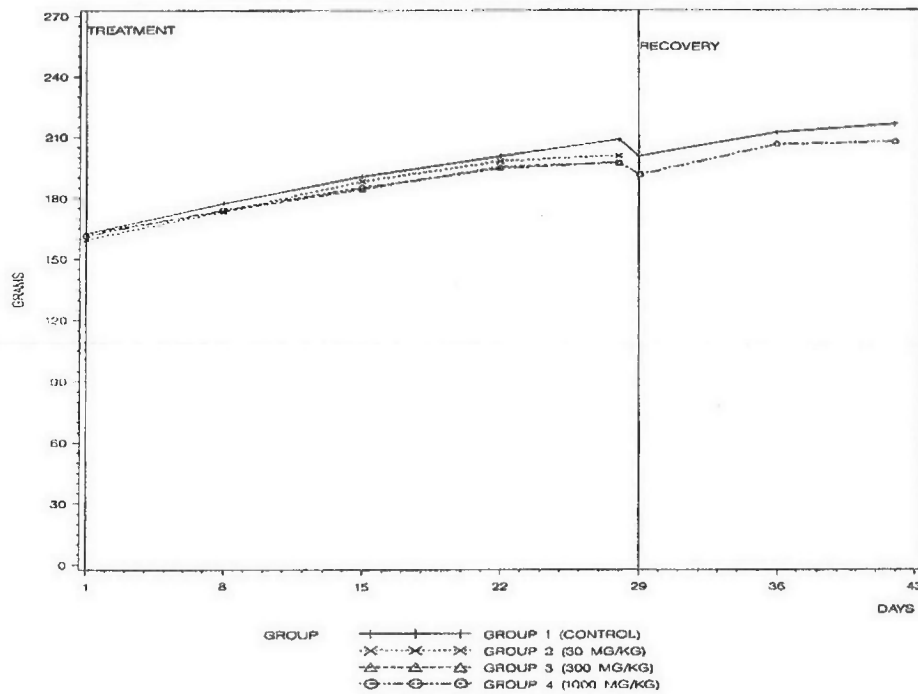
FIGURES AND SUMMARY TABLES

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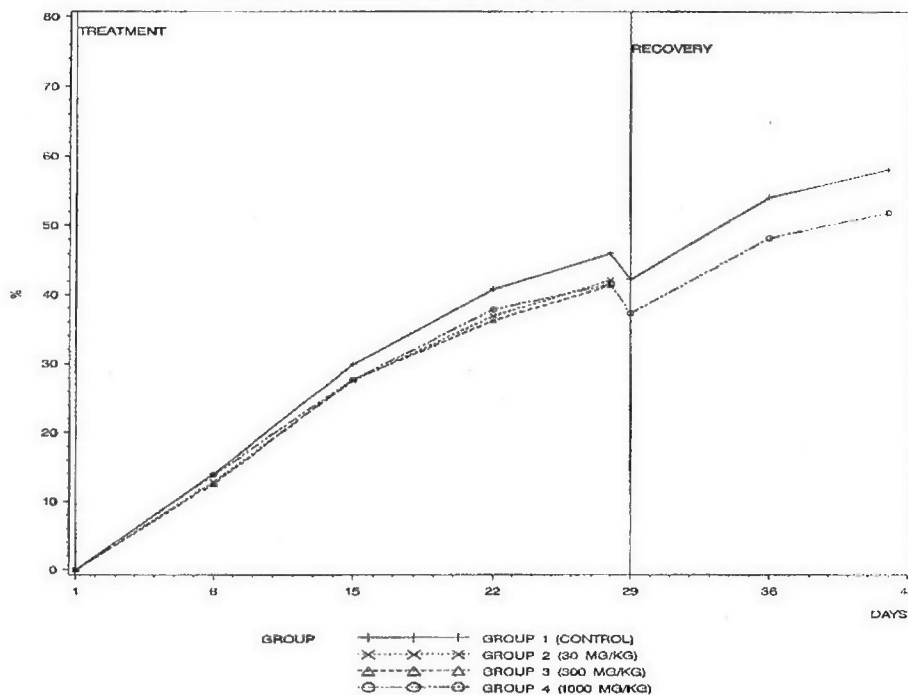
BODY WEIGHTS (GRAM) MALES



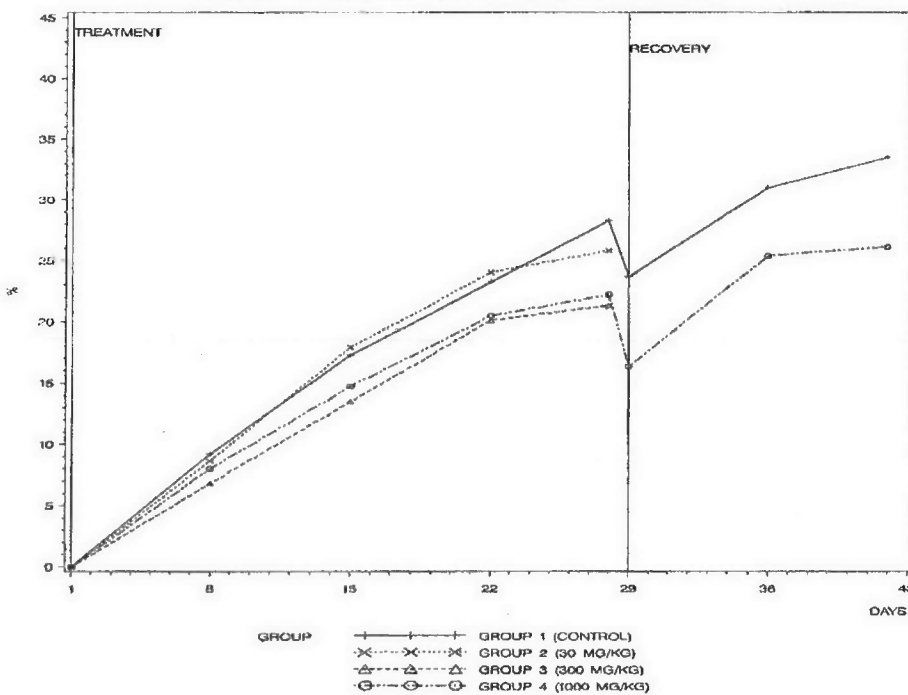
BODY WEIGHTS (GRAM) FEMALES

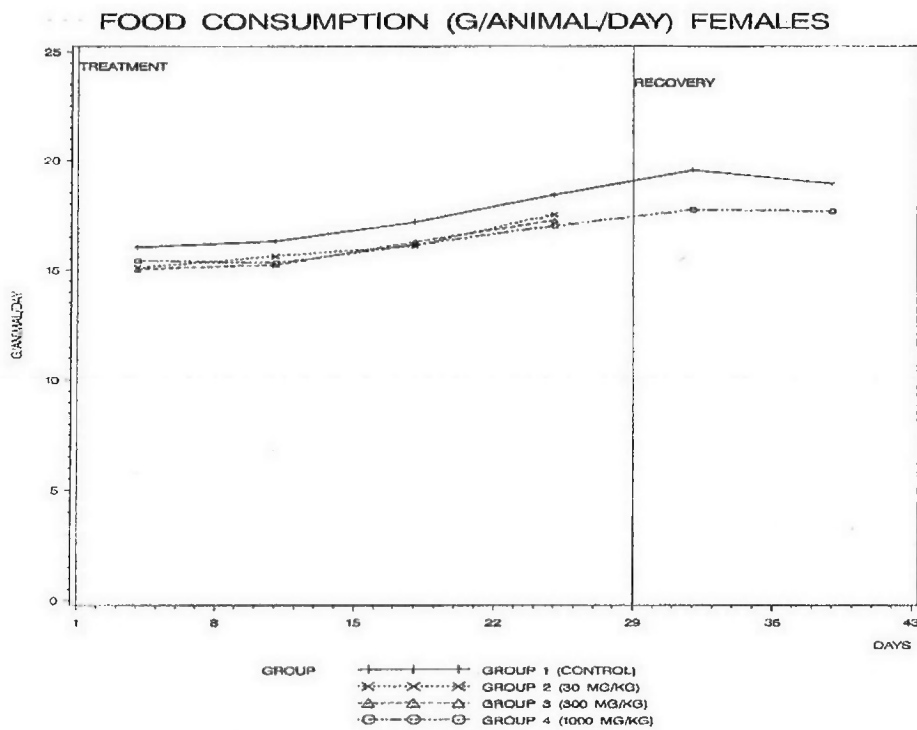
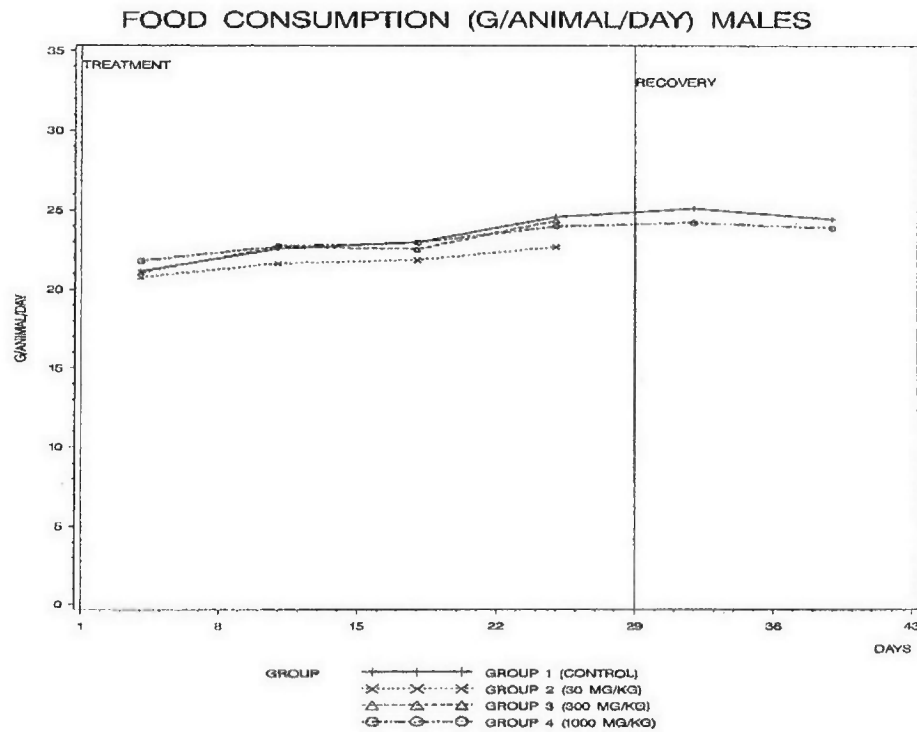


BODY WEIGHT GAIN (%) MALES



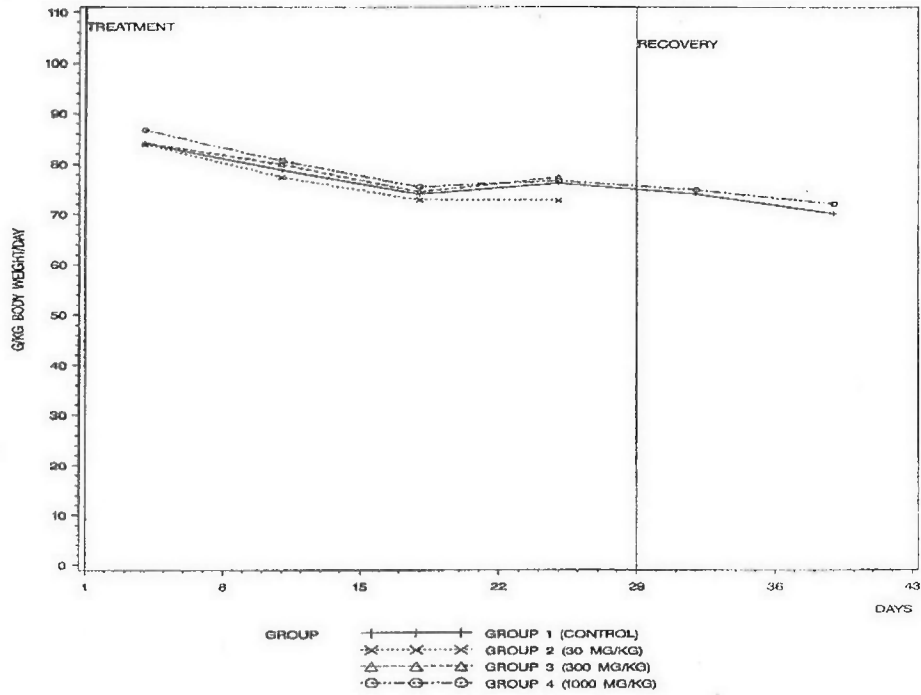
BODY WEIGHT GAIN (%) FEMALES



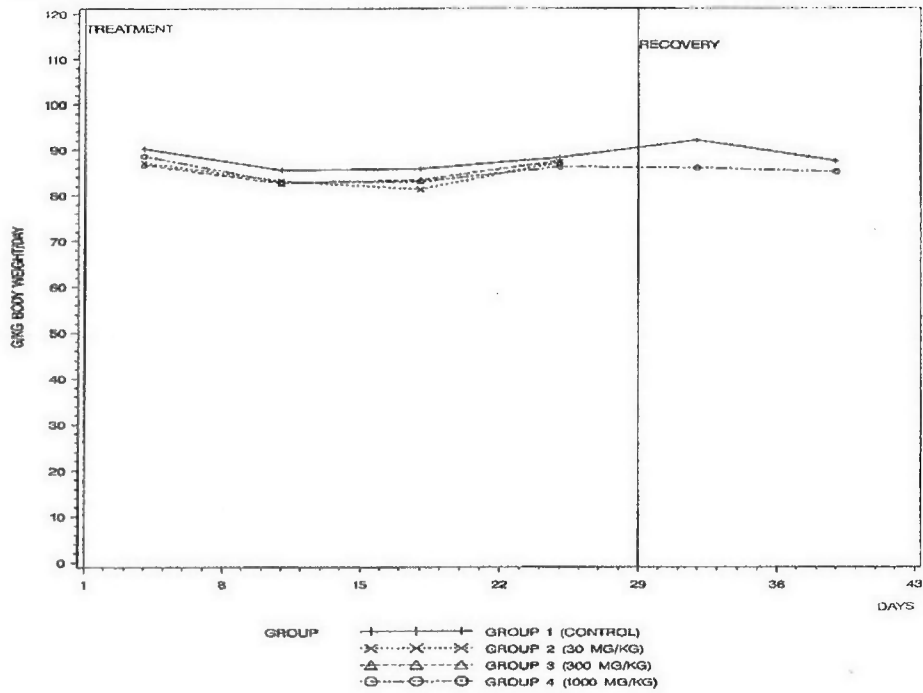


APPENDIX 1

RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) MALES



RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) FEMALES



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CLINICAL SIGNS SUMMARY MALES

	TREATMENT	RECOVERY
SIGN (MAX. GRADE)	WEEK: 1..... 4.....	1.....
(LOCATION)	DAY: 12345671234567123456712345671234567	

GROUP 1 (CONTROL)

Secretion / excretion		
Chromodacryorrhoea (3)	G:	1.....
(Periorbital region right)	%:	2.....

GROUP 2 (30 MG/KG)

Secretion / excretion		
Salivation (3)	G:	111
	%:	666

GROUP 3 (300 MG/KG)

Secretion / excretion		
Salivation (3)	G:	111111111111
	%:	644AA4424288

GROUP 4 (1000 MG/KG)

Secretion / excretion		
Salivation (3)	G: . 11111111. 1111111111111111.	
	%: . 1245AAAA A99A62AA5775666.	

FEMALES

	TREATMENT	RECOVERY
SIGN (MAX. GRADE)	WEEK: 1..... 4.....	1.....
(LOCATION)	DAY: 12345671234567123456712345671234567	

GROUP 1 (CONTROL)

Secretion / excretion		
Salivation (3)	G:	1.....
	%:	1.....

GROUP 2 (30 MG/KG)

Secretion / excretion		
Salivation (3)	G:	1111. 111
	%:	4222. 224

GROUP 3 (300 MG/KG)

Skin / fur		
Erythema maculate (4)	G:	111. . .
(Ear right)	%:	222. . .
Secretion / excretion		
Salivation (3)	G:	1. . . 1. . . . 1
	%:	6. . . 2. . . . 2

GROUP 4 (1000 MG/KG)

Secretion / excretion		
Salivation (3)	G: . 1. 11111111. . . 111111111111. . . 1.	
	%: . 1. 114AA811. . . 334A33AA144. . . 1.	
Various		
Opacity (1)	G:	11.
(Eye right)	%:	11.
Dark (3)	G:	111.
(Eye right)	%:	222.

G: Median value of the highest individual daily grades
%: Percent of affected animals (0=less than 5%, 1=between 5% and 15%,..., A=more than 95%)
.: Observation performed, sign not present

APPENDIX 1

**FUNCTIONAL OBSERVATIONS SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
AT WEEK 4					
HEARING	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
PUPIL L	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
PUPIL R	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
STATIC R	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
GRIP	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10

FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
AT WEEK 4					
HEARING	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
PUPIL L	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
PUPIL R	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
STATIC R	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10
GRIP	MEDIAN	0	0	0	0
SCORE 0/1	N	10	5	5	10

+ / ++ Steel-test significant at 5% (+) or 1% (++) level

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MOTOR ACTIVITY MEASUREMENTS SUMMARY
MALES
WEEK 4

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
total high sensor count	MEAN	2300	1144 *	960 **	2683
	ST.DEV	648	454	344	930
	N	9	5	5	10
total low sensor count	MEAN	4237	3868	3842	4598
	ST.DEV	822	1031	806	742
	N	10	5	5	10

FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
total high sensor count	MEAN	1691	1223	843	1547
	ST.DEV	813	360	576	832
	N	10	5	5	10
total low sensor count	MEAN	4300	4069	3641	4630
	ST.DEV	1706	649	2288	1951
	N	10	5	5	10

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

**BODY WEIGHTS (GRAM) SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAY 1	MEAN	220	219	222	221
WEEK 1	ST.DEV	8.0	6.0	5.6	9.1
	N	10	5	5	10
DAY 8	MEAN	251	247	250	251
WEEK 2	ST.DEV	12.0	6.5	8.1	10.9
	N	10	5	5	10
DAY 15	MEAN	286	280	284	282
WEEK 3	ST.DEV	18.6	6.1	9.4	11.1
	N	10	5	5	10
DAY 22	MEAN	311	300	303	304
WEEK 4	ST.DEV	23.5	4.7	10.4	12.1
	N	10	5	5	10
DAY 28	MEAN	322	311	314	312
WEEK 4	ST.DEV	26.1	5.0	14.7	14.9
	N	10	5	5	10
RECOVERY					
DAY 1	MEAN	313			299
WEEK 1	ST.DEV	26.6			12.8
	N	5			5
DAY 8	MEAN	339			323
WEEK 2	ST.DEV	34.3			13.1
	N	5			5
DAY 14	MEAN	348			331
WEEK 2	ST.DEV	39.5			13.7
	N	5			5

FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAY 1	MEAN	163	160	163	162
WEEK 1	ST.DEV	5.0	7.0	6.2	8.6
	N	10	5	5	10
DAY 8	MEAN	178	173	174	174
WEEK 2	ST.DEV	5.9	5.6	7.0	7.2
	N	10	5	5	10
DAY 15	MEAN	191	188	184	185
WEEK 3	ST.DEV	7.8	9.2	6.1	6.6
	N	10	5	5	10
DAY 22	MEAN	200	198	195	195
WEEK 4	ST.DEV	7.3	10.8	5.9	11.4
	N	10	5	5	10
DAY 28	MEAN	209	201	197	197 *
WEEK 4	ST.DEV	7.3	9.3	7.8	10.6
	N	10	5	5	10

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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**BODY WEIGHTS (GRAM) SUMMARY
FEMALES**

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		CONTROL	30 MG/KG	300 MG/KG	1000 MG/KG
RECOVERY					
DAY 1	MEAN	200			191
WEEK 1	ST.DEV	9.9			9.2
	N	5			5
DAY 8	MEAN	212			206
WEEK 2	ST.DEV	9.9			9.8
	N	5			5
DAY 14	MEAN	216			207
WEEK 2	ST.DEV	7.4			9.0
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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APPENDIX 1

**BODY WEIGHT GAIN (%) SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAY 1	MEAN	0	0	0	0
WEEK 1	ST.DEV	0.0	0.0	0.0	0.0
	N	10	5	5	10
DAY 8	MEAN	14	13	13	14
WEEK 2	ST.DEV	1.9	1.2	1.8	4.6
	N	10	5	5	10
DAY 15	MEAN	30	28	28	28
WEEK 3	ST.DEV	4.4	2.1	2.3	5.3
	N	10	5	5	10
DAY 22	MEAN	41	37	36	38
WEEK 4	ST.DEV	6.2	3.4	2.7	5.2
	N	10	5	5	10
DAY 28	MEAN	46	42	41	42
WEEK 4	ST.DEV	7.1	3.4	3.8	6.5
	N	10	5	5	10
RECOVERY					
DAY 1	MEAN	42			37
WEEK 1	ST.DEV	7.6			6.6
	N	5			5
DAY 8	MEAN	54			48
WEEK 2	ST.DEV	10.5			7.5
	N	5			5
DAY 14	MEAN	58			52
WEEK 2	ST.DEV	12.7			8.2
	N	5			5

FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAY 1	MEAN	0	0	0	0
WEEK 1	ST.DEV	0.0	0.0	0.0	0.0
	N	10	5	5	10
DAY 8	MEAN	9	9	7	8
WEEK 2	ST.DEV	3.0	1.3	4.7	3.8
	N	10	5	5	10
DAY 15	MEAN	17	18	13	15
WEEK 3	ST.DEV	4.4	2.5	4.6	3.5
	N	10	5	5	10
DAY 22	MEAN	23	24	20	20
WEEK 4	ST.DEV	3.5	3.0	3.1	4.5
	N	10	5	5	10
DAY 28	MEAN	28	26	21 **	22 **
WEEK 4	ST.DEV	3.9	2.8	3.7	3.9
	N	10	5	5	10

/ * Dunnett-test based on pooled variance significant at 5% (*) or 1% () level

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**BODY WEIGHT GAIN (%) SUMMARY
FEMALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
RECOVERY					
DAY 1	MEAN	24			16 *
WEEK 1	ST.DEV	4.0			3.4
	N	5			5
DAY 8	MEAN	31			25
WEEK 2	ST.DEV	4.0			5.3
	N	5			5
DAY 14	MEAN	33			26 *
WEEK 2	ST.DEV	2.4			5.2
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
MALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAYS 1-8	MEAN	21	21	21	22
WEEKS 1-2	ST.DEV	0.3	---	---	0.8
	N (CAGE)	2	1	1	2
DAYS 8-15	MEAN	23	22	23	23
WEEKS 2-3	ST.DEV	0.2	---	---	0.6
	N (CAGE)	2	1	1	2
DAYS 15-22	MEAN	23	22	23	23
WEEKS 3-4	ST.DEV	0.3	---	---	1.1
	N (CAGE)	2	1	1	2
DAYS 22-28	MEAN	25	23	24	24
WEEK 4	ST.DEV	0.7	---	---	1.9
	N (CAGE)	2	1	1	2
MEAN OF MEANS OVER TREATMENT MEAN		23	22	23	23
RECOVERY					
DAYS 1-8	MEAN	25			24
WEEKS 1-2	ST.DEV	---			---
	N (CAGE)	1			1
DAYS 8-14	MEAN	24			24
WEEK 2	ST.DEV	---			---
	N (CAGE)	1			1
MEAN OF MEANS OVER RECOVERY MEAN		25			24

FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAYS 1-8	MEAN	16	15	15	15
WEEKS 1-2	ST.DEV	0.3	---	---	0.4
	N (CAGE)	2	1	1	2
DAYS 8-15	MEAN	16	16	15	15
WEEKS 2-3	ST.DEV	0.1	---	---	0.3
	N (CAGE)	2	1	1	2
DAYS 15-22	MEAN	17	16	16	16
WEEKS 3-4	ST.DEV	0.2	---	---	0.2
	N (CAGE)	2	1	1	2
DAYS 22-28	MEAN	18	18	17	17
WEEK 4	ST.DEV	0.0	---	---	0.5
	N (CAGE)	2	1	1	2
MEAN OF MEANS OVER TREATMENT MEAN		17	16	16	16
RECOVERY					
DAYS 1-8	MEAN	20			18
WEEKS 1-2	ST.DEV	---			---
	N (CAGE)	1			1
DAYS 8-14	MEAN	19			18
WEEK 2	ST.DEV	---			---
	N (CAGE)	1			1

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**FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY
FEMALES**

	GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
RECOVERY MEAN OF MEANS OVER RECOVERY MEAN	19			18

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**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAYS 1-8	MEAN	84	84	84	87
WEEKS 1-2	ST.DEV	0.5	---	---	0.9
	N (CAGE)	2	1	1	2
DAYS 8-15	MEAN	79	77	80	81
WEEKS 2-3	ST.DEV	0.2	---	---	0.1
	N (CAGE)	2	1	1	2
DAYS 15-22	MEAN	74	73	74	75
WEEKS 3-4	ST.DEV	0.6	---	---	1.8
	N (CAGE)	2	1	1	2
DAYS 22-28	MEAN	76	73	77	77
WEEK 4	ST.DEV	1.7	---	---	3.9
	N (CAGE)	2	1	1	2
MEAN OF MEANS OVER TREATMENT MEAN		78	77	79	80
RECOVERY					
DAYS 1-8	MEAN	74			75
WEEKS 1-2	ST.DEV	---			---
	N (CAGE)	1			1
DAYS 8-14	MEAN	70			72
WEEK 2	ST.DEV	---			---
	N (CAGE)	1			1
MEAN OF MEANS OVER RECOVERY MEAN		72			74

FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
TREATMENT					
DAYS 1-8	MEAN	90	87	87	89
WEEKS 1-2	ST.DEV	0.9	---	---	1.5
	N (CAGE)	2	1	1	2
DAYS 8-15	MEAN	86	83	83	83
WEEKS 2-3	ST.DEV	2.8	---	---	2.6
	N (CAGE)	2	1	1	2
DAYS 15-22	MEAN	86	81	83	83
WEEKS 3-4	ST.DEV	2.9	---	---	0.6
	N (CAGE)	2	1	1	2
DAYS 22-28	MEAN	88	87	88	86
WEEK 4	ST.DEV	1.3	---	---	0.6
	N (CAGE)	2	1	1	2
MEAN OF MEANS OVER TREATMENT MEAN		88	85	85	85
RECOVERY					
DAYS 1-8	MEAN	92			86
WEEKS 1-2	ST.DEV	---			---
	N (CAGE)	1			1
DAYS 8-14	MEAN	88			85
WEEK 2	ST.DEV	---			---
	N (CAGE)	1			1

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**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY) SUMMARY
FEMALES**

	GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
RECOVERY MEAN OF MEANS OVER RECOVERY MEAN	90			86

APPENDIX 1

HAEMATOLOGY SUMMARY
MALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
WBC	MEAN	10.6	10.2	10.6	11.8
10E9/L	ST.DEV	2.8	3.6	2.1	1.5
	N	10	5	5	10
Neutrophils	MEAN	13.6	9.7	12.5	13.5
%WBC	ST.DEV	3.5	1.9	1.6	2.6
	N	10	5	5	10
Lymphocytes	MEAN	83.5	87.4	84.7	83.6
%WBC	ST.DEV	3.5	1.2	2.1	2.9
	N	10	5	5	10
Monocytes	MEAN	1.8	1.8	1.6	1.9
%WBC	ST.DEV	0.9	1.3	0.6	1.1
	N	10	5	5	10
Eosinophils	MEAN	0.8	0.8	0.7	0.6
%WBC	ST.DEV	0.2	0.7	0.3	0.3
	N	10	5	5	10
Basophils	MEAN	0.3	0.3	0.4	0.3
%WBC	ST.DEV	0.2	0.2	0.2	0.2
	N	10	5	5	10
Red blood cells	MEAN	8.71	8.29	8.47	8.36
10E12/L	ST.DEV	0.54	0.19	0.36	0.44
	N	10	5	5	10
Reticulocytes	MEAN	3.1	2.8	3.5	3.7
%RBC	ST.DEV	0.4	0.5	0.3	0.6
	N	10	5	5	10
RDW	MEAN	13.2	13.2	12.2	12.8
%	ST.DEV	2.8	3.6	0.2	0.7
	N	10	5	5	10
Haemoglobin	MEAN	9.9	9.4 *	9.6	9.3 **
mmol/L	ST.DEV	0.4	0.2	0.3	0.4
	N	10	5	5	10
Haematocrit	MEAN	0.452	0.427	0.443	0.434
L/L	ST.DEV	0.024	0.014	0.015	0.018
	N	10	5	5	10
MCV	MEAN	52.0	51.5	52.3	51.8
fL	ST.DEV	0.6	0.9	1.1	1.0
	N	10	5	5	10
MCH	MEAN	1.14	1.13	1.13	1.12
fmol	ST.DEV	0.04	0.02	0.04	0.03
	N	10	5	5	10
MCHC	MEAN	21.85	21.95	21.63	21.55
mmol/L	ST.DEV	0.52	0.33	0.52	0.36
	N	10	5	5	10
Platelets	MEAN	981	1000	1014	1018
10E9/L	ST.DEV	124	60	148	126
	N	10	5	5	10
PT	MEAN	17.0	17.5	15.5	16.8
s	ST.DEV	1.6	0.6	0.6	1.1
	N	10	5	5	10
APTT	MEAN	16.2	17.1	14.5	16.9
s	ST.DEV	2.8	1.7	3.3	1.3
	N	9	5	5	10

+/** Steel-test significant at 5% (+) or 1% (**) level

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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**HAEMATOLOGY SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
WBC	MEAN	9.5			10.0
10E9/L	ST.DEV	3.4			1.6
	N	3			5
Neutrophils	MEAN	15.0			16.8
%WBC	ST.DEV	6.6			5.8
	N	3			5
Lymphocytes	MEAN	80.8			79.5
%WBC	ST.DEV	7.0			6.1
	N	3			5
Monocytes	MEAN	2.3			2.2
%WBC	ST.DEV	0.2			0.5
	N	3			5
Eosinophils	MEAN	1.4			1.0
%WBC	ST.DEV	0.2			0.3
	N	3			5
Basophils	MEAN	0.4			0.4
%WBC	ST.DEV	0.1			0.0
	N	3			5
Red blood cells	MEAN	8.60			8.31
10E12/L	ST.DEV	0.70			0.10
	N	3			5
Reticulocytes	MEAN	2.9			3.7 +
%RBC	ST.DEV	0.3			0.5
	N	3			5
RDW	MEAN	15.1			14.7
%	ST.DEV	3.8			0.5
	N	3			5
Haemoglobin	MEAN	9.9			9.5
mmol/L	ST.DEV	0.5			0.2
	N	3			5
Haematocrit	MEAN	0.444			0.442
L/L	ST.DEV	0.024			0.014
	N	3			5
MCV	MEAN	51.7			53.2
fL	ST.DEV	1.5			1.5
	N	3			5
MCH	MEAN	1.15			1.14
fmol	ST.DEV	0.05			0.03
	N	3			5
MCHC	MEAN	22.20			21.40 *
mmol/L	ST.DEV	0.35			0.36
	N	3			5
Platelets	MEAN	1013			956
10E9/L	ST.DEV	213			76
	N	3			5
PT	MEAN	17.3			17.5
s	ST.DEV	1.3			0.6
	N	3			4
APTT	MEAN	16.3			17.9
s	ST.DEV	3.2			2.8
	N	3			4

+ / + + Steel-test significant at 5% (+) or 1% (++) level

* / ** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

HAEMATOLOGY SUMMARY
FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
WBC	MEAN	5.5	4.9	6.6	7.4
10E9/L	ST.DEV	1.6	1.7	1.5	2.5
	N	10	5	5	10
Neutrophils	MEAN	13.4	13.9	13.0	11.0
%WBC	ST.DEV	3.8	2.2	5.5	3.2
	N	10	5	5	10
Lymphocytes	MEAN	83.3	83.8	84.3	86.9
%WBC	ST.DEV	4.4	2.4	5.8	3.6
	N	10	5	5	10
Monocytes	MEAN	1.5	1.3	1.9	1.2
%WBC	ST.DEV	0.3	0.6	0.9	0.4
	N	10	5	5	10
Eosinophils	MEAN	1.5	0.8 ++	0.5 ++	0.6 ++
%WBC	ST.DEV	0.6	0.2	0.3	0.4
	N	10	5	5	10
Basophils	MEAN	0.3	0.2	0.3	0.3
%WBC	ST.DEV	0.1	0.0	0.2	0.1
	N	10	5	5	10
Red blood cells	MEAN	8.00	7.83	8.35	8.06
10E12/L	ST.DEV	0.38	0.32	0.36	0.42
	N	10	5	5	10
Reticulocytes	MEAN	3.4	3.8	3.8	3.4
%RBC	ST.DEV	0.5	0.5	0.8	0.8
	N	10	5	5	10
RDW	MEAN	11.5	11.8	11.9	11.6
%	ST.DEV	0.6	0.6	0.7	0.6
	N	10	5	5	10
Haemoglobin	MEAN	9.1	8.8	9.4	8.9
mmol/L	ST.DEV	0.4	0.3	0.5	0.4
	N	10	5	5	10
Haematocrit	MEAN	0.420	0.397	0.431	0.408
L/L	ST.DEV	0.019	0.020	0.025	0.025
	N	10	5	5	10
MCV	MEAN	52.5	50.7	51.6	50.6 *
fL	ST.DEV	1.4	1.2	1.8	1.6
	N	10	5	5	10
MCH	MEAN	1.14	1.13	1.13	1.10 *
fmol	ST.DEV	0.03	0.02	0.03	0.02
	N	10	5	5	10
MCHC	MEAN	21.62	22.26	21.86	21.84
mmol/L	ST.DEV	0.42	0.54	0.68	0.46
	N	10	5	5	10
Platelets	MEAN	1106	869 **	946	900 **
10E9/L	ST.DEV	121	125	90	135
	N	10	5	5	10
PT	MEAN	16.8	16.6	16.4	16.6
s	ST.DEV	0.5	0.8	1.2	0.6
	N	10	5	5	10
APTT	MEAN	17.0	15.3	17.3	17.3
s	ST.DEV	1.9	1.5	2.9	2.3
	N	10	5	5	10

+ / + + Steel-test significant at 5% (+) or 1% (++) level

* / ** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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**HAEMATOLOGY SUMMARY
FEMALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
WBC	MEAN	5.0			5.2
10E9/L	ST.DEV	1.7			1.9
	N	5			5
Neutrophils	MEAN	12.4			11.9
%WBC	ST.DEV	6.0			3.1
	N	5			5
Lymphocytes	MEAN	83.4			84.8
%WBC	ST.DEV	7.1			3.8
	N	5			5
Monocytes	MEAN	2.1			1.8
%WBC	ST.DEV	1.0			0.3
	N	5			5
Eosinophils	MEAN	1.6			1.2
%WBC	ST.DEV	0.5			0.9
	N	5			5
Basophils	MEAN	0.5			0.3
%WBC	ST.DEV	0.1			0.1
	N	5			5
Red blood cells	MEAN	8.20			7.74
10E12/L	ST.DEV	0.39			0.68
	N	5			5
Reticulocytes	MEAN	2.4			2.9
%RBC	ST.DEV	0.2			0.6
	N	5			5
RDW	MEAN	12.5			14.3 **
%	ST.DEV	0.2			0.8
	N	5			5
Haemoglobin	MEAN	9.5			9.0
mmol/L	ST.DEV	0.4			0.9
	N	5			5
Haematocrit	MEAN	0.430			0.409
L/L	ST.DEV	0.026			0.046
	N	5			5
MCV	MEAN	52.3			52.7
fL	ST.DEV	0.8			1.8
	N	5			5
MCH	MEAN	1.16			1.16
fmol	ST.DEV	0.01			0.03
	N	5			5
MCHC	MEAN	22.18			22.06
mmol/L	ST.DEV	0.36			0.42
	N	5			5
Platelets	MEAN	1123			877 *
10E9/L	ST.DEV	105			128
	N	5			5
PT	MEAN	17.8			16.6
s	ST.DEV	1.0			1.1
	N	5			4
APTT	MEAN	17.9			16.9
s	ST.DEV	2.3			2.4
	N	5			4

+ / + + Steel-test significant at 5% (+) or 1% (++) level

* / ** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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APPENDIX 1

CLINICAL BIOCHEMISTRY SUMMARY
MALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
ALAT	MEAN	51.3	38.8	44.9	39.9
U/L	ST.DEV	11.5	7.4	17.8	7.0
	N	10	5	5	10
ASAT	MEAN	74.7	74.3	76.6	77.4
U/L	ST.DEV	9.5	4.9	2.7	9.4
	N	10	5	5	10
ALP	MEAN	172	140	173	142
U/L	ST.DEV	50	33	42	35
	N	10	5	5	10
Total protein	MEAN	64.9	62.3	65.6	65.2
g/L	ST.DEV	2.5	2.8	1.4	2.4
	N	10	5	5	10
Albumin	MEAN	32.5	31.5	32.4	32.4
g/L	ST.DEV	1.3	0.9	0.8	1.3
	N	10	5	5	10
Total bilirubin	MEAN	2.3	1.9 *	1.7 **	1.9 **
umol/L	ST.DEV	0.4	0.2	0.1	0.2
	N	10	5	5	10
Urea	MEAN	8.3	8.1	8.0	8.6
mmol/L	ST.DEV	1.1	1.7	1.8	1.4
	N	10	5	5	10
Creatinine	MEAN	40.9	40.1	40.5	42.0
umol/L	ST.DEV	1.3	2.4	2.4	2.5
	N	10	5	5	10
Glucose	MEAN	8.74	8.80	9.09	9.46
mmol/L	ST.DEV	1.93	1.26	2.80	2.38
	N	10	5	5	10
Cholesterol	MEAN	1.98	2.03	2.38	1.98
mmol/L	ST.DEV	0.47	0.24	0.48	0.36
	N	10	5	5	10
Bile Acids	MEAN	44.0	50.7	25.3 *	29.4 *
umol/l	ST.DEV	11.9	15.7	9.3	6.9
	N	10	5	5	10
Sodium	MEAN	141.6	140.9	141.6	141.5
mmol/L	ST.DEV	0.6	1.3	1.5	1.0
	N	10	5	5	10
Potassium	MEAN	4.15	4.00	4.34	4.15
mmol/L	ST.DEV	0.18	0.33	0.22	0.24
	N	10	5	5	10
Chloride	MEAN	104	103	104	104
mmol/L	ST.DEV	1	1	1	1
	N	10	5	5	10
Calcium	MEAN	2.83	2.80	2.82	2.84
mmol/L	ST.DEV	0.04	0.07	0.05	0.06
	N	10	5	5	10
Inorg.Phos	MEAN	2.15	2.17	2.18	2.19
mmol/L	ST.DEV	0.15	0.16	0.12	0.10
	N	10	5	5	10

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

**CLINICAL BIOCHEMISTRY SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
ALAT	MEAN	42.5			45.7
U/L	ST.DEV	8.7			15.2
	N	5			5
ASAT	MEAN	75.1			80.4
U/L	ST.DEV	8.4			19.5
	N	5			5
ALP	MEAN	161			137
U/L	ST.DEV	35			18
	N	5			5
Total protein	MEAN	64.5			63.8
g/L	ST.DEV	2.0			1.8
	N	5			5
Albumin	MEAN	32.5			32.6
g/L	ST.DEV	1.0			0.8
	N	5			5
Total bilirubin	MEAN	2.3			2.2
umol/L	ST.DEV	0.3			0.2
	N	5			5
Urea	MEAN	7.2			8.4
mmol/L	ST.DEV	0.7			2.1
	N	5			5
Creatinine	MEAN	42.3			42.6
umol/L	ST.DEV	1.1			1.4
	N	5			5
Glucose	MEAN	11.11			11.92
mmol/L	ST.DEV	1.24			3.67
	N	5			5
Cholesterol	MEAN	1.86			1.71
mmol/L	ST.DEV	0.43			0.31
	N	5			5
Bile Acids	MEAN	28.6			28.2
umol/l	ST.DEV	7.4			11.5
	N	5			5
Sodium	MEAN	141.8			141.4
mmol/L	ST.DEV	0.5			1.2
	N	5			5
Potassium	MEAN	4.01			4.44
mmol/L	ST.DEV	0.31			0.28
	N	5			5
Chloride	MEAN	103			104
mmol/L	ST.DEV	1			2
	N	5			5
Calcium	MEAN	2.83			2.89
mmol/L	ST.DEV	0.06			0.06
	N	5			5
Inorg.Phos	MEAN	1.97			2.28 **
mmol/L	ST.DEV	0.13			0.14
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

CLINICAL BIOCHEMISTRY SUMMARY
FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
ALAT U/L	MEAN	39.7	42.9	36.4	34.2
	ST.DEV	10.6	15.1	8.5	11.0
	N	10	5	5	10
ASAT U/L	MEAN	71.5	78.8	75.5	79.6
	ST.DEV	7.0	11.7	7.9	10.0
	N	10	5	5	10
ALP U/L	MEAN	76	70	56	65
	ST.DEV	17	28	4	9
	N	10	5	5	10
Total protein g/L	MEAN	65.9	66.5	68.7	67.3
	ST.DEV	2.9	2.5	1.5	3.4
	N	10	5	5	10
Albumin g/L	MEAN	34.4	33.9	35.8	34.7
	ST.DEV	1.7	1.2	0.6	1.9
	N	10	5	5	10
Total bilirubin umol/L	MEAN	2.1	2.4	2.1	2.2
	ST.DEV	0.2	0.6	0.2	0.3
	N	10	5	5	10
Urea mmol/L	MEAN	8.9	9.3	8.6	9.4
	ST.DEV	2.0	0.8	1.0	1.6
	N	10	5	5	10
Creatinine umol/L	MEAN	43.9	44.7	42.6	43.3
	ST.DEV	1.9	1.1	0.7	3.1
	N	10	5	5	10
Glucose mmol/L	MEAN	7.56	7.31	6.43	6.94
	ST.DEV	0.72	1.16	0.62	0.94
	N	10	5	5	10
Cholesterol mmol/L	MEAN	1.59	1.98	2.27 *	2.32 **
	ST.DEV	0.35	0.41	0.26	0.56
	N	10	5	5	10
Bile Acids umol/l	MEAN	23.6	37.3	27.2	28.9
	ST.DEV	8.8	13.6	13.2	9.9
	N	10	5	5	10
Sodium mmol/L	MEAN	141.1	140.4	141.1	140.6
	ST.DEV	1.5	0.5	1.0	1.2
	N	10	5	5	10
Potassium mmol/L	MEAN	3.55	3.48	3.64	3.71
	ST.DEV	0.22	0.26	0.24	0.20
	N	10	5	5	10
Chloride mmol/L	MEAN	106	104	105	105
	ST.DEV	1	2	1	2
	N	10	5	5	10
Calcium mmol/L	MEAN	2.78	2.74	2.80	2.78
	ST.DEV	0.10	0.10	0.13	0.12
	N	10	5	5	10
Inorg.Phos mmol/L	MEAN	1.91	1.70	1.82	1.81
	ST.DEV	0.31	0.10	0.17	0.23
	N	10	5	5	10

** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

CLINICAL BIOCHEMISTRY SUMMARY FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
ALAT	MEAN	38.9			33.6
U/L	ST.DEV	7.4			9.0
	N	5			5
ASAT	MEAN	73.3			87.1
U/L	ST.DEV	6.2			23.5
	N	5			5
ALP	MEAN	71			61
U/L	ST.DEV	21			11
	N	5			5
Total protein	MEAN	66.3			65.3
g/L	ST.DEV	2.3			2.8
	N	5			5
Albumin	MEAN	34.0			34.7
g/L	ST.DEV	1.3			1.4
	N	5			5
Total bilirubin	MEAN	2.2			2.3
umol/L	ST.DEV	0.2			0.3
	N	5			5
Urea	MEAN	7.5			8.6
mmol/L	ST.DEV	1.1			1.4
	N	5			5
Creatinine	MEAN	44.0			48.6 *
umol/L	ST.DEV	2.0			2.8
	N	5			5
Glucose	MEAN	8.93			8.22
mmol/L	ST.DEV	1.22			1.71
	N	5			5
Cholesterol	MEAN	1.70			1.58
mmol/L	ST.DEV	0.38			0.38
	N	5			5
Bile Acids	MEAN	20.8			13.8
umol/l	ST.DEV	7.3			3.8
	N	5			5
Sodium	MEAN	140.8			140.6
mmol/L	ST.DEV	0.6			1.0
	N	5			5
Potassium	MEAN	3.37			3.62 *
mmol/L	ST.DEV	0.05			0.19
	N	5			5
Chloride	MEAN	104			104
mmol/L	ST.DEV	1			2
	N	5			5
Calcium	MEAN	2.79			2.75
mmol/L	ST.DEV	0.05			0.03
	N	5			5
Inorg.Phos	MEAN	1.59			1.74
mmol/L	ST.DEV	0.24			0.17
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

**MACROSCOPIC FINDINGS SUMMARY
MALES**

	GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT				
Animals examined	5	5	5	5
Animals without findings	4	5	5	5
Animals affected	1	0	0	0
Seminal vesicles				
Reduced in size	1	0	0	0
END OF RECOVERY				
Animals examined	5			5
Animals without findings	5			5

FEMALES

	GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT				
Animals examined	5	5	5	5
Animals without findings	4	5	4	4
Animals affected	1	0	1	1
Uterus				
Contains fluid	1	0	1	1
END OF RECOVERY				
Animals examined	5			5
Animals without findings	2			3
Animals affected	3			2
Uterus				
Contains fluid	1			2
Clitoral glands				
Focus/foci	1			0
Discolouration	1			0

/ ## Fisher's Exact test significant at 5% (#) or 1% (##) level

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**ORGAN WEIGHTS (GRAM) SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
BODY W. (GRAM)	MEAN	306	291	292	296
	ST.DEV	26	8	12	13
	N	5	5	5	5
BRAIN (GRAM)	MEAN	1.96	1.92	1.97	1.89
	ST.DEV	0.07	0.07	0.06	0.09
	N	5	5	5	5
HEART (GRAM)	MEAN	0.865	0.893	0.889	0.879
	ST.DEV	0.063	0.072	0.038	0.075
	N	5	5	5	5
LIVER (GRAM)	MEAN	8.19	8.04	8.85	9.08
	ST.DEV	0.91	0.49	0.26	0.70
	N	5	5	5	5
THYROIDES (GRAM)	MEAN	0.025	0.030	0.028	0.032
	ST.DEV	0.005	0.007	0.006	0.007
	N	5	5	5	5
THYMUS (GRAM)	MEAN	0.439	0.405	0.378	0.377
	ST.DEV	0.060	0.068	0.048	0.114
	N	5	5	5	5
KIDNEYS (GRAM)	MEAN	2.23	2.18	2.31	2.34
	ST.DEV	0.26	0.13	0.21	0.21
	N	5	5	5	5
ADRENALS (GRAM)	MEAN	0.054	0.065	0.060	0.067
	ST.DEV	0.004	0.009	0.005	0.011
	N	5	5	5	5
SPLEEN (GRAM)	MEAN	0.599	0.567	0.627	0.629
	ST.DEV	0.084	0.081	0.043	0.050
	N	5	5	5	5
TESTES (GRAM)	MEAN	3.07	3.36	3.23	3.20
	ST.DEV	0.33	0.39	0.04	0.26
	N	5	5	5	5
PROSTATE (GRAM)	MEAN	0.442	0.485	0.516	0.463
	ST.DEV	0.106	0.102	0.103	0.063
	N	5	5	5	5
EPIDIDYMIDES (GRAM)	MEAN	0.871	0.899	0.882	0.934
	ST.DEV	0.066	0.075	0.054	0.039
	N	5	5	5	5
SEMINAL VES (GRAM)	MEAN	0.897	0.848	0.960	1.108
	ST.DEV	0.187	0.106	0.157	0.141
	N	5	5	5	5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

ORGAN WEIGHTS (GRAM) SUMMARY
MALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
BODY W. (GRAM)	MEAN	332			315
	ST.DEV	38			13
	N	5			5
BRAIN (GRAM)	MEAN	1.95			1.89
	ST.DEV	0.07			0.07
	N	5			5
HEART (GRAM)	MEAN	0.963			0.970
	ST.DEV	0.080			0.093
	N	5			5
LIVER (GRAM)	MEAN	8.34			8.42
	ST.DEV	1.04			0.44
	N	5			5
THYROIDS (GRAM)	MEAN	0.028			0.024
	ST.DEV	0.004			0.008
	N	5			5
THYMUS (GRAM)	MEAN	0.394			0.395
	ST.DEV	0.077			0.096
	N	5			5
KIDNEYS (GRAM)	MEAN	2.33			2.26
	ST.DEV	0.21			0.28
	N	5			5
ADRENALS (GRAM)	MEAN	0.061			0.064
	ST.DEV	0.008			0.013
	N	5			5
SPLEEN (GRAM)	MEAN	0.584			0.611
	ST.DEV	0.084			0.063
	N	5			5
TESTES (GRAM)	MEAN	3.25			3.17
	ST.DEV	0.39			0.30
	N	5			5
PROSTATE (GRAM)	MEAN	0.514			0.550
	ST.DEV	0.072			0.078
	N	5			5
EPIDIDYMIDES (GRAM)	MEAN	0.978			1.022
	ST.DEV	0.077			0.059
	N	5			5
SEMINAL VES (GRAM)	MEAN	1.298			1.187
	ST.DEV	0.210			0.067
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

**ORGAN/BODY WEIGHT RATIOS (%) SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
BODY W. (GRAM)	MEAN	306	291	292	296
	ST.DEV	26	8	12	13
	N	5	5	5	5
BRAIN (%)	MEAN	0.64	0.66	0.68	0.64
	ST.DEV	0.04	0.03	0.03	0.05
	N	5	5	5	5
HEART (%)	MEAN	0.283	0.308	0.304	0.297
	ST.DEV	0.009	0.031	0.016	0.017
	N	5	5	5	5
LIVER (%)	MEAN	2.67	2.76	3.03 **	3.06 **
	ST.DEV	0.10	0.10	0.06	0.13
	N	5	5	5	5
THYROIDES (%)	MEAN	0.008	0.010	0.010	0.011
	ST.DEV	0.002	0.003	0.002	0.002
	N	5	5	5	5
THYMUS (%)	MEAN	0.143	0.140	0.129	0.127
	ST.DEV	0.015	0.026	0.015	0.035
	N	5	5	5	5
KIDNEYS (%)	MEAN	0.73	0.75	0.79	0.79
	ST.DEV	0.03	0.03	0.09	0.05
	N	5	5	5	5
ADRENALS (%)	MEAN	0.018	0.022	0.021	0.023
	ST.DEV	0.003	0.003	0.002	0.004
	N	5	5	5	5
SPLEEN (%)	MEAN	0.195	0.196	0.215	0.213
	ST.DEV	0.019	0.033	0.016	0.013
	N	5	5	5	5
TESTES (%)	MEAN	1.00	1.15 *	1.11	1.08
	ST.DEV	0.08	0.14	0.04	0.05
	N	5	5	5	5
PROSTATE (%)	MEAN	0.144	0.166	0.177	0.157
	ST.DEV	0.030	0.032	0.039	0.024
	N	5	5	5	5
EPIDIDYMIDES (%)	MEAN	0.286	0.309	0.302	0.315
	ST.DEV	0.025	0.029	0.017	0.004
	N	5	5	5	5
SEMINAL VES (%)	MEAN	0.293	0.291	0.328	0.376 *
	ST.DEV	0.056	0.030	0.054	0.056
	N	5	5	5	5

** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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**ORGAN/BODY WEIGHT RATIOS (%) SUMMARY
MALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
BODY W. (GRAM)	MEAN	332			315
	ST.DEV	38			13
	N	5			5
BRAIN (%)	MEAN	0.59			0.60
	ST.DEV	0.05			0.03
	N	5			5
HEART (%)	MEAN	0.291			0.307
	ST.DEV	0.017			0.019
	N	5			5
LIVER (%)	MEAN	2.51			2.67
	ST.DEV	0.15			0.10
	N	5			5
THYROIDS (%)	MEAN	0.009			0.008
	ST.DEV	0.001			0.002
	N	5			5
THYMUS (%)	MEAN	0.118			0.125
	ST.DEV	0.016			0.028
	N	5			5
KIDNEYS (%)	MEAN	0.70			0.71
	ST.DEV	0.04			0.07
	N	5			5
ADRENALS (%)	MEAN	0.018			0.020
	ST.DEV	0.001			0.004
	N	5			5
SPLEEN (%)	MEAN	0.177			0.194
	ST.DEV	0.027			0.020
	N	5			5
TESTES (%)	MEAN	0.98			1.01
	ST.DEV	0.08			0.09
	N	5			5
PROSTATE (%)	MEAN	0.157			0.174
	ST.DEV	0.031			0.020
	N	5			5
EPIDIDYMIDES (%)	MEAN	0.296			0.324
	ST.DEV	0.026			0.024
	N	5			5
SEMINAL VES (%)	MEAN	0.396			0.376
	ST.DEV	0.086			0.013
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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APPENDIX 1

ORGAN WEIGHTS (GRAM) SUMMARY
FEMALES

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
BODY W. (GRAM)	MEAN	189	183	179	175
	ST.DEV	6	12	6	14
	N	5	5	5	5
BRAIN (GRAM)	MEAN	1.75	1.79	1.77	1.75
	ST.DEV	0.04	0.06	0.05	0.08
	N	5	5	5	5
HEART (GRAM)	MEAN	0.680	0.642	0.633	0.617
	ST.DEV	0.054	0.051	0.082	0.046
	N	5	5	5	5
LIVER (GRAM)	MEAN	5.55	5.67	5.97	5.75
	ST.DEV	0.21	0.21	0.52	0.13
	N	5	5	5	5
THYROIDS (GRAM)	MEAN	0.026	0.024	0.025	0.032
	ST.DEV	0.005	0.003	0.005	0.005
	N	5	5	5	5
THYMUS (GRAM)	MEAN	0.370	0.294	0.286 *	0.316
	ST.DEV	0.052	0.031	0.045	0.061
	N	5	5	5	5
KIDNEYS (GRAM)	MEAN	1.53	1.49	1.49	1.49
	ST.DEV	0.08	0.09	0.12	0.10
	N	5	5	5	5
ADRENALS (GRAM)	MEAN	0.069	0.075	0.070	0.071
	ST.DEV	0.011	0.007	0.010	0.013
	N	5	5	5	5
SPLEEN (GRAM)	MEAN	0.421	0.463	0.425	0.393
	ST.DEV	0.057	0.022	0.077	0.010
	N	5	5	5	5
OVARIES (GRAM)	MEAN	0.121	0.138	0.125	0.125
	ST.DEV	0.018	0.012	0.027	0.008
	N	5	5	5	5
UTERUS (GRAM)	MEAN	0.447	0.430	0.477	0.449
	ST.DEV	0.152	0.086	0.195	0.093
	N	5	5	5	5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

**ORGAN WEIGHTS (GRAM) SUMMARY
FEMALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
BODY W. (GRAM)	MEAN	203			195
	ST.DEV	7			8
	N	5			5
BRAIN (GRAM)	MEAN	1.79			1.82
	ST.DEV	0.04			0.05
	N	5			5
HEART (GRAM)	MEAN	0.732			0.665
	ST.DEV	0.080			0.049
	N	5			5
LIVER (GRAM)	MEAN	5.47			5.31
	ST.DEV	0.37			0.47
	N	5			5
THYROIDS (GRAM)	MEAN	0.030			0.026
	ST.DEV	0.004			0.002
	N	5			5
THYMUS (GRAM)	MEAN	0.353			0.358
	ST.DEV	0.047			0.066
	N	5			5
KIDNEYS (GRAM)	MEAN	1.51			1.46
	ST.DEV	0.07			0.15
	N	5			5
ADRENALS (GRAM)	MEAN	0.076			0.070
	ST.DEV	0.017			0.012
	N	5			5
SPLEEN (GRAM)	MEAN	0.478			0.461
	ST.DEV	0.038			0.068
	N	5			5
OVARIES (GRAM)	MEAN	0.133			0.129
	ST.DEV	0.014			0.023
	N	5			5
UTERUS (GRAM)	MEAN	0.546			0.489
	ST.DEV	0.225			0.138
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

APPENDIX 1

**ORGAN/BODY WEIGHT RATIOS (%) SUMMARY
FEMALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF TREATMENT					
BODY W. (GRAM)	MEAN	189	183	179	175
	ST.DEV	6	12	6	14
	N	5	5	5	5
BRAIN (%)	MEAN	0.92	0.98	0.99	1.01
	ST.DEV	0.02	0.06	0.04	0.10
	N	5	5	5	5
HEART (%)	MEAN	0.359	0.351	0.353	0.355
	ST.DEV	0.020	0.014	0.038	0.043
	N	5	5	5	5
LIVER (%)	MEAN	2.93	3.11	3.33 *	3.30 *
	ST.DEV	0.16	0.17	0.20	0.28
	N	5	5	5	5
THYROIDES (%)	MEAN	0.014	0.013	0.014	0.019 *
	ST.DEV	0.003	0.002	0.003	0.004
	N	5	5	5	5
THYMUS (%)	MEAN	0.196	0.161	0.160	0.180
	ST.DEV	0.029	0.015	0.025	0.029
	N	5	5	5	5
KIDNEYS (%)	MEAN	0.81	0.82	0.84	0.85
	ST.DEV	0.03	0.05	0.07	0.05
	N	5	5	5	5
ADRENALS (%)	MEAN	0.037	0.042	0.039	0.041
	ST.DEV	0.005	0.006	0.006	0.009
	N	5	5	5	5
SPLEEN (%)	MEAN	0.223	0.254	0.237	0.225
	ST.DEV	0.031	0.019	0.036	0.014
	N	5	5	5	5
OVARIES (%)	MEAN	0.064	0.076	0.070	0.072
	ST.DEV	0.009	0.008	0.017	0.009
	N	5	5	5	5
UTERUS (%)	MEAN	0.237	0.236	0.267	0.260
	ST.DEV	0.084	0.051	0.110	0.068
	N	5	5	5	5

/ Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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**ORGAN/BODY WEIGHT RATIOS (%) SUMMARY
FEMALES**

		GROUP 1 CONTROL	GROUP 2 30 MG/KG	GROUP 3 300 MG/KG	GROUP 4 1000 MG/KG
END OF RECOVERY					
BODY W. (GRAM)	MEAN	203			195
	ST.DEV	7			8
	N	5			5
BRAIN (%)	MEAN	0.88			0.94 *
	ST.DEV	0.03			0.04
	N	5			5
HEART (%)	MEAN	0.360			0.342
	ST.DEV	0.031			0.016
	N	5			5
LIVER (%)	MEAN	2.69			2.73
	ST.DEV	0.13			0.16
	N	5			5
THYROIDS (%)	MEAN	0.015			0.013
	ST.DEV	0.002			0.001
	N	5			5
THYMUS (%)	MEAN	0.174			0.183
	ST.DEV	0.024			0.028
	N	5			5
KIDNEYS (%)	MEAN	0.74			0.75
	ST.DEV	0.04			0.06
	N	5			5
ADRENALS (%)	MEAN	0.037			0.036
	ST.DEV	0.008			0.005
	N	5			5
SPLEEN (%)	MEAN	0.235			0.236
	ST.DEV	0.016			0.029
	N	5			5
OVARIES (%)	MEAN	0.066			0.066
	ST.DEV	0.006			0.010
	N	5			5
UTERUS (%)	MEAN	0.269			0.250
	ST.DEV	0.110			0.061
	N	5			5

*/** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

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APPENDIX 2

INDIVIDUAL DATA TABLES

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**MORTALITY DATA
MALES**

ANIMAL	SCHEDULED SACRIFICE	TREATMENT FROM	TO
GROUP 1 (CONTROL)			
1	10APR09	13MAR09	09APR09
2	10APR09	13MAR09	09APR09
3	10APR09	13MAR09	09APR09
4	10APR09	13MAR09	09APR09
5	10APR09	13MAR09	09APR09
6	24APR09	13MAR09	09APR09
7	24APR09	13MAR09	09APR09
8	24APR09	13MAR09	09APR09
9	24APR09	13MAR09	09APR09
10	24APR09	13MAR09	09APR09
GROUP 2 (30 MG/KG)			
11	10APR09	13MAR09	09APR09
12	10APR09	13MAR09	09APR09
13	10APR09	13MAR09	09APR09
14	10APR09	13MAR09	09APR09
15	10APR09	13MAR09	09APR09
GROUP 3 (300 MG/KG)			
16	10APR09	13MAR09	09APR09
17	10APR09	13MAR09	09APR09
18	10APR09	13MAR09	09APR09
19	10APR09	13MAR09	09APR09
20	10APR09	13MAR09	09APR09
GROUP 4 (1000 MG/KG)			
21	10APR09	13MAR09	09APR09
22	10APR09	13MAR09	09APR09
23	10APR09	13MAR09	09APR09
24	10APR09	13MAR09	09APR09
25	10APR09	13MAR09	09APR09
26	24APR09	13MAR09	09APR09
27	24APR09	13MAR09	09APR09
28	24APR09	13MAR09	09APR09
29	24APR09	13MAR09	09APR09
30	24APR09	13MAR09	09APR09

FEMALES

ANIMAL	SCHEDULED SACRIFICE	TREATMENT FROM	TO
GROUP 1 (CONTROL)			
31	10APR09	13MAR09	09APR09
32	10APR09	13MAR09	09APR09
33	10APR09	13MAR09	09APR09
34	10APR09	13MAR09	09APR09
35	10APR09	13MAR09	09APR09
36	24APR09	13MAR09	09APR09
37	24APR09	13MAR09	09APR09
38	24APR09	13MAR09	09APR09
39	24APR09	13MAR09	09APR09
40	24APR09	13MAR09	09APR09
GROUP 2 (30 MG/KG)			
41	10APR09	13MAR09	09APR09
42	10APR09	13MAR09	09APR09
43	10APR09	13MAR09	09APR09
44	10APR09	13MAR09	09APR09
45	10APR09	13MAR09	09APR09

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APPENDIX 2

**MORTALITY DATA
FEMALES**

ANIMAL SCHEDULED SACRIFICE		TREATMENT FROM	TO
<hr/>			
GROUP 3 (300 MG/KG)			
46	10APR09	13MAR09	09APR09
47	10APR09	13MAR09	09APR09
48	10APR09	13MAR09	09APR09
49	10APR09	13MAR09	09APR09
50	10APR09	13MAR09	09APR09
GROUP 4 (1000 MG/KG)			
51	10APR09	13MAR09	09APR09
52	10APR09	13MAR09	09APR09
53	10APR09	13MAR09	09APR09
54	10APR09	13MAR09	09APR09
55	10APR09	13MAR09	09APR09
56	24APR09	13MAR09	09APR09
57	24APR09	13MAR09	09APR09
58	24APR09	13MAR09	09APR09
59	24APR09	13MAR09	09APR09
60	24APR09	13MAR09	09APR09

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**CLINICAL SIGNS
MALES**

SIGN (MAX. GRADE) (LOCATION)	TREATMENT				RECOVERY			
	WEEK: 1.....	4.....	1.....		WEEK: 1.....	4.....	1.....	
	DAY: 1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567
GROUP 1 (CONTROL)								
ANIMAL 1								
No clinical signs noted								
ANIMAL 2								
No clinical signs noted								
ANIMAL 3								
No clinical signs noted								
ANIMAL 4								
No clinical signs noted								
ANIMAL 5								
No clinical signs noted								
ANIMAL 6								
No clinical signs noted								
ANIMAL 7								
No clinical signs noted								
ANIMAL 8								
Secretion / excretion								
Chromodacryorrhoea (3)	G:	1.....					
(Periorbital region right)								
ANIMAL 9								
No clinical signs noted								
ANIMAL 10								
No clinical signs noted								
GROUP 2 (30 MG/KG)								
ANIMAL 11								
Secretion / excretion								
Salivation (3)	G:	11					
ANIMAL 12								
Secretion / excretion								
Salivation (3)	G:	111					
ANIMAL 13								
Secretion / excretion								
Salivation (3)	G:	111					
ANIMAL 14								
No clinical signs noted								
ANIMAL 15								
Secretion / excretion								
Salivation (3)	G:	1..					
GROUP 3 (300 MG/KG)								
ANIMAL 16								
Secretion / excretion								
Salivation (3)	G:	1.. 11... 1111					
ANIMAL 17								
Secretion / excretion								
Salivation (3)	G:	1111... 1. 11					
ANIMAL 18								
Secretion / excretion								
Salivation (3)	G:	11111... 11					
ANIMAL 19								
Secretion / excretion								
Salivation (3)	G:	1.. 11111...					
ANIMAL 20								
Secretion / excretion								
Salivation (3)	G:	1111... 11					
GROUP 4 (1000 MG/KG)								
ANIMAL 21								
Secretion / excretion								
Salivation (3)	G:	... 111111... 11111111... 1111						

G: Highest daily grades
 .. Observation performed, sign not present

APPENDIX 2

**CLINICAL SIGNS
MALES**

SIGN (MAX. GRADE) (LOCATION)	TREATMENT		RECOVERY	
	WEEK: 1.....	4.....	1.....	
	DAY: 12345671234567123456712345671234567			
GROUP 4 (1000 MG/KG)				
ANIMAL 22				
Secretion / excretion				
Salivation (3)	G:	1111.	1111. 11111.	
ANIMAL 23				
Secretion / excretion				
Salivation (3)	G:	1111.	11111. 11111. 111	
ANIMAL 24				
Secretion / excretion				
Salivation (3)	G:	1111.	11111. 11. 111.	
ANIMAL 25				
Secretion / excretion				
Salivation (3)	G: ...	111111.	11. 111111111. 111	
ANIMAL 26				
Secretion / excretion				
Salivation (3)	G: ..	11111111.	1111. 11. ... 1.	
ANIMAL 27				
Secretion / excretion				
Salivation (3)	G:	111111.	111111. 11. 1111111.	
ANIMAL 28				
Secretion / excretion				
Salivation (3)	G:	1111.	1111. 111111. 111.	
ANIMAL 29				
Secretion / excretion				
Salivation (3)	G:	1111.	1111. 11.	
ANIMAL 30				
Secretion / excretion				
Salivation (3)	G: ..	111111111.	1. 112. 1111111111.	

FEMALES

SIGN (MAX. GRADE) (LOCATION)	TREATMENT		RECOVERY	
	WEEK: 1.....	4.....	1.....	
	DAY: 12345671234567123456712345671234567			
GROUP 1 (CONTROL)				
ANIMAL 31				
No clinical signs noted				
ANIMAL 32				
No clinical signs noted				
ANIMAL 33				
No clinical signs noted				
ANIMAL 34				
No clinical signs noted				
ANIMAL 35				
Secretion / excretion				
Salivation (3)	G:	1.		
ANIMAL 36				
No clinical signs noted				
ANIMAL 37				
No clinical signs noted				
ANIMAL 38				
No clinical signs noted				
ANIMAL 39				
No clinical signs noted				
ANIMAL 40				
No clinical signs noted				

G: Highest daily grades
.: Observation performed, sign not present

CLINICAL SIGNS FEMALES

SIGN (MAX. GRADE) (LOCATION)	TREATMENT				RECOVERY			
	WEEK: 1.....	2.....	3.....	4.....	1.....	2.....	3.....	4.....
	DAY: 1234567	1234567	1234567	1234567	1234567	1234567	1234567	1234567
GROUP 2 (30 MG/KG)								
ANIMAL 41								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 42								
No clinical signs noted								
ANIMAL 43								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 44								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 45								
Secretion / excretion								
Salivation (3)	G:
GROUP 3 (300 MG/KG)								
ANIMAL 46								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 47								
No clinical signs noted								
ANIMAL 48								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 49								
Skin / fur								
Erythema maculate (4) (Ear right)	G:
Secretion / excretion								
Salivation (3)	G:
ANIMAL 50								
No clinical signs noted								
GROUP 4 (1000 MG/KG)								
ANIMAL 51								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 52								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 53								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 54								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 55								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 56								
Secretion / excretion								
Salivation (3)	G:
ANIMAL 57								
Secretion / excretion								
Salivation (3)	G:
Various								
Dark (3) (Eye right)	G:

G: Highest daily grades
.: Observation performed, sign not present

APPENDIX 2

**CLINICAL SIGNS
FEMALES**

	TREATMENT	RECOVERY
SIGN (MAX. GRADE)	WEEK: 1.....4.....1.....	
(LOCATION)	DAY: 12345671234567123456712345671234567	

GROUP 4 (1000 MG/KG)**ANIMAL 58**

Secretion / excretion

Salivation (3)

G: ... 11. 111..... 11111111.....

ANIMAL 59

Secretion / excretion

Salivation (3)

G: 111..... 111.. 11.....

Various

Opacity (1)

G: 11.....

(Eye right)

ANIMAL 60

Secretion / excretion

Salivation (3)

G: 111..... 11.. 11.....

G: Highest daily grades
 ∴ Observation performed, sign not present

APPENDIX 2

**FUNCTIONAL OBSERVATIONS
MALES
AT WEEK 4**

ANIMAL	HEARING SCORE 0/1	PUPIL L SCORE 0/1	PUPIL R SCORE 0/1	STATIC R SCORE 0/1	GRIP SCORE 0/1
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GROUP 1 (CONTROL)

1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0

GROUP 2 (30 MG/KG)

11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0

GROUP 3 (300 MG/KG)

16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0

GROUP 4 (1000 MG/KG)

21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0
28	0	0	0	0	0
29	0	0	0	0	0
30	0	0	0	0	0

**FEMALES
AT WEEK 4**

ANIMAL	HEARING SCORE 0/1	PUPIL L SCORE 0/1	PUPIL R SCORE 0/1	STATIC R SCORE 0/1	GRIP SCORE 0/1
--------	----------------------	----------------------	----------------------	-----------------------	-------------------

GROUP 1 (CONTROL)

31	0	0	0	0	0
32	0	0	0	0	0
33	0	0	0	0	0
34	0	0	0	0	0
35	0	0	0	0	0
36	0	0	0	0	0
37	0	0	0	0	0
38	0	0	0	0	0
39	0	0	0	0	0
40	0	0	0	0	0

APPENDIX 2

**FUNCTIONAL OBSERVATIONS
FEMALES
AT WEEK 4**

ANIMAL	HEARING SCORE 0/1	PUPIL L SCORE 0/1	PUPIL R SCORE 0/1	STATIC R SCORE 0/1	GRIP SCORE 0/1
--------	----------------------	----------------------	----------------------	-----------------------	-------------------

GROUP 2 (30 MG/KG)

41	0	0	0	0	0
42	0	0	0	0	0
43	0	0	0	0	0
44	0	0	0	0	0
45	0	0	0	0	0

GROUP 3 (300 MG/KG)

46	0	0	0	0	0
47	0	0	0	0	0
48	0	0	0	0	0
49	0	0	0	0	0
50	0	0	0	0	0

GROUP 4 (1000 MG/KG)

51	0	0	0	0	0
52	0	0	0	0	0
53	0	0	0	0	0
54	0	0	0	0	0
55	0	0	0	0	0
56	0	0	0	0	0
57	0	0	0	0	0
58	0	0	0	0	0
59	0	0	0	0	0
60	0	0	0	0	0

MOTOR ACTIVITY TEST
MALES
WEEK 4

Animal number	Location of sensor	Counts per sample period (hour)												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
GROUP 1 (CONTROL)														
1	High	78	30	6	64	191	172	103	170	334	188	346	419	2101
1	Low	224	114	177	462	496	409	379	406	536	377	455	767	4802
2	High *	516	1637	281	4602	1622	8364	2595	206	222	178	106	80	20409
2	Low	281	109	502	347	283	257	402	408	523	364	324	292	4092
3	High	32	65	71	296	161	99	103	285	145	244	114	89	1704
3	Low	184	293	290	696	359	308	264	532	544	347	347	363	4527
4	High	63	206	93	432	487	249	35	315	285	88	191	140	2584
4	Low	201	377	266	495	639	146	79	313	242	338	320	367	3783
5	High	50	39	93	389	102	128	149	172	102	90	111	5	1430
5	Low	551	442	587	551	162	333	527	434	305	423	535	52	4902
6	High	238	116	771	854	208	25	54	169	326	269	348	299	3677
6	Low	287	190	104	666	318	43	100	106	347	168	97	330	2756
7	High	151	21	198	303	343	215	173	226	346	246	200	70	2492
7	Low	314	398	325	220	410	120	246	309	493	421	437	134	3827
8	High	94	52	95	258	331	212	256	178	341	234	277	86	2414
8	Low	102	187	205	413	420	160	342	333	389	212	564	160	3487
9	High	1	203	63	376	225	539	73	323	39	126	310	134	2412
9	Low	128	745	654	707	524	623	90	587	123	269	844	353	5647
10	High	41	47	166	310	296	356	193	320	9	55	82	11	1886
10	Low	327	171	363	372	708	499	271	514	276	349	400	299	4549
GROUP 2 (30 MG/KG)														
11	High	21	136	98	223	0	0	10	145	98	153	83	47	1014
11	Low	56	668	492	655	21	2	39	222	552	142	192	185	3226
12	High	38	18	34	94	55	159	1	12	35	94	93	42	675
12	Low	144	69	163	393	212	548	0	190	220	238	473	171	2821
13	High	59	45	34	192	366	99	2	148	19	192	369	274	1799
13	Low	606	392	344	548	823	222	54	420	268	537	697	578	5489
14	High	8	35	117	263	71	20	133	19	17	105	39	11	838
14	Low	154	488	726	832	309	149	248	401	92	384	316	45	4144
15	High	42	2	41	165	200	204	63	17	40	321	166	135	1396
15	Low	218	145	258	307	280	517	186	82	266	479	686	236	3660
GROUP 3 (300 MG/KG)														
16	High	47	38	180	66	20	42	26	55	0	163	40	120	797
16	Low	260	369	455	227	255	75	117	351	1	416	204	506	3236
17	High	0	90	143	70	1	179	3	180	102	58	34	25	885
17	Low	88	700	516	361	81	644	93	371	377	332	301	266	4130
18	High	14	123	7	94	123	31	144	0	7	33	69	107	752
18	Low	264	851	116	668	405	128	414	34	198	248	258	355	3939
19	High	59	30	74	188	97	228	61	83	168	143	251	188	1570
19	Low	462	324	368	320	261	765	216	268	672	293	640	392	4981
20	High	56	21	123	75	94	149	0	4	2	170	81	21	796
20	Low	262	52	217	257	304	403	5	46	136	672	501	68	2923

* Considered to be an outlier; excluded from statistical analyses.

**MOTOR ACTIVITY TEST
MALES
WEEK 4**

Animal number	Location of sensor	Counts per sample period (hour)											Total	
		1	2	3	4	5	6	7	8	9	10	11		12
GROUP 4 (1000 MG/KG)														
21	High	136	51	84	236	272	255	89	265	38	54	79	154	1713
21	Low	229	257	162	335	381	480	99	760	64	141	179	178	3265
22	High	163	175	133	307	355	391	248	29	69	261	242	248	2621
22	Low	123	202	305	650	348	297	287	71	295	343	338	404	3663
23	High	56	27	38	474	424	449	316	215	285	57	111	156	2608
23	Low	121	101	258	509	586	424	443	355	650	201	420	304	4372
24	High	20	15	50	157	127	490	139	304	21	151	536	371	2381
24	Low	135	237	273	289	368	488	246	708	23	441	705	1063	4976
25	High	74	36	270	490	494	245	0	19	100	314	328	63	2433
25	Low	242	323	595	668	872	400	0	202	250	701	837	270	5360
26	High	141	53	62	212	150	440	199	32	12	230	176	51	1758
26	Low	254	142	216	370	474	576	516	94	66	653	637	302	4300
27	High	273	104	106	283	474	432	187	608	447	347	359	235	3855
27	Low	501	145	227	465	491	492	224	612	615	562	551	481	5366
28	High	161	88	126	246	146	123	248	55	291	326	11	305	2126
28	Low	491	82	490	532	446	195	625	123	578	864	247	847	5520
29	High	363	9	90	572	349	572	107	67	266	55	98	75	2623
29	Low	464	19	352	743	577	629	338	296	478	109	278	292	4575
30	High	214	62	52	291	297	318	375	365	726	734	749	532	4715
30	Low	450	190	332	316	649	301	400	196	402	385	770	187	4578

**MOTOR ACTIVITY TEST
FEMALES
WEEK 4**

Animal number	Location of sensor	Counts per sample period (hour)												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
GROUP 1 (CONTROL)														
31	High	116	51	193	148	122	55	66	166	71	92	156	169	1405
31	Low	205	216	556	441	620	213	346	515	391	234	619	489	4845
32	High	51	117	65	229	181	350	153	82	114	83	175	224	1824
32	Low	329	283	276	470	492	608	406	161	351	223	364	360	4323
33	High	84	24	183	291	83	156	47	70	20	70	66	96	1190
33	Low	330	90	454	620	126	512	85	267	306	71	486	248	3595
34	High	98	4	55	143	243	188	46	93	49	102	94	113	1228
34	Low	301	22	254	297	621	559	77	174	381	307	300	146	3439
35	High	22	45	134	351	427	102	256	142	44	81	147	3	1754
35	Low	160	125	472	591	657	232	470	474	161	227	488	25	4082
36	High	39	12	82	177	260	47	67	22	24	48	136	24	938
36	Low	155	74	322	377	514	335	175	83	83	130	221	151	2620
37	High	51	74	182	105	101	13	127	138	30	61	22	34	938
37	Low	100	278	517	248	299	57	495	491	371	126	113	131	3226
38	High	237	293	334	361	426	339	319	228	202	166	142	164	3211
38	Low	478	632	445	731	664	503	644	428	560	424	361	484	6354
39	High	109	193	263	244	311	282	333	154	277	245	204	431	3046
39	Low	576	453	465	637	821	566	657	650	799	657	720	957	7958
40	High	85	136	42	212	330	85	60	16	30	121	60	201	1378
40	Low	301	217	78	280	499	187	177	84	99	172	158	302	2554
GROUP 2 (30 MG/KG)														
41	High	81	34	37	230	80	16	39	203	72	153	93	28	1066
41	Low	403	182	220	402	357	55	243	470	179	827	285	149	3772
42	High	55	88	119	157	60	250	74	111	53	149	110	90	1316
42	Low	175	366	268	286	204	408	280	282	135	292	249	190	3135
43	High	187	154	173	294	85	129	162	47	115	130	242	94	1812
43	Low	579	346	321	485	356	314	230	124	223	461	375	423	4237
44	High	147	13	51	252	47	91	15	21	101	57	88	49	932
44	Low	590	235	191	932	129	260	192	194	639	441	341	199	4343
45	High	57	52	184	205	36	8	84	0	78	61	218	7	990
45	Low	370	382	662	772	383	92	505	53	314	592	516	216	4857
GROUP 3 (300 MG/KG)														
46	High	29	150	43	63	35	48	0	0	37	14	14	22	455
46	Low	151	307	202	419	93	401	1	60	226	109	245	63	2277
47	High	97	3	39	40	73	95	12	54	40	66	50	118	687
47	Low	616	5	382	401	283	415	21	130	159	554	316	438	3720
48	High	198	64	33	190	33	200	51	88	47	248	275	383	1810
48	Low	744	481	511	609	523	948	388	604	503	564	501	1120	7496
49	High	56	5	4	14	9	47	91	30	17	3	30	75	381
49	Low	145	50	49	148	51	137	288	171	182	84	175	204	1684
50	High	35	100	93	35	22	112	85	90	75	56	40	139	882
50	Low	200	77	168	225	255	191	262	502	172	189	379	407	3027

**MOTOR ACTIVITY TEST
MALES
WEEK 4**

Animal number	Location of sensor	Counts per sample period (hour)												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
GROUP 4 (1000 MG/KG)														
51	High	121	62	144	244	234	226	193	115	135	165	170	229	2038
51	Low	281	160	331	362	427	363	372	168	258	442	329	541	4034
52	High	77	151	125	157	119	45	251	119	17	75	89	88	1313
52	Low	337	387	205	331	504	80	372	296	31	113	290	250	3196
53	High	243	135	102	135	187	138	252	164	193	127	222	231	2129
53	Low	384	327	207	374	392	326	678	445	434	355	555	426	4903
54	High	384	214	292	296	276	234	262	215	217	164	168	283	3005
54	Low	456	566	510	418	406	417	564	689	724	508	386	641	6285
55	High	217	33	55	482	664	394	227	54	66	198	114	46	2550
55	Low	701	193	439	1236	922	908	400	538	510	748	562	222	7379
56	High	46	123	7	60	156	52	0	62	0	71	66	12	655
56	Low	82	344	142	269	378	140	12	468	0	440	356	39	2670
57	High	118	19	40	148	69	76	82	2	36	82	131	76	879
57	Low	511	57	321	417	277	430	653	16	207	465	766	425	4545
58	High	109	13	11	149	161	231	117	114	126	1	259	49	1340
58	Low	653	230	400	925	966	908	773	318	747	318	914	490	7642
59	High	108	44	126	90	51	20	82	0	55	2	73	86	737
59	Low	295	117	175	211	282	11	215	4	201	43	63	197	1814
60	High	68	53	84	88	32	69	112	96	16	35	117	54	824
60	Low	220	326	304	301	233	330	399	299	105	277	564	477	3835

APPENDIX 2

**BODY WEIGHTS (GRAM)
MALES**

	TREATMENT					RECOVERY		
DAYS	1	8	15	22	28	1	8	14
WEEKS	1	2	3	4	4	1	2	2
ANIMAL								

GROUP 1 (CONTROL)

1	206	230	258	275	282	---	---	---
2	227	255	284	310	325	---	---	---
3	218	253	284	308	311	---	---	---
4	224	260	298	325	343	---	---	---
5	230	268	314	341	356	---	---	---
6	216	242	275	295	308	303	323	326
7	232	268	316	351	363	356	397	414
8	215	247	279	304	317	307	333	342
9	215	243	268	285	292	284	307	311
10	220	248	288	311	322	314	335	347

GROUP 2 (30 MG/KG)

11	225	256	288	305	316			
12	215	242	274	294	309			
13	211	241	276	301	310			
14	221	246	276	296	305			
15	224	252	284	303	317			

GROUP 3 (300 MG/KG)

16	227	259	295	314	334			
17	221	249	287	307	315			
18	225	257	287	307	318			
19	213	239	270	287	293			
20	225	247	280	298	311			

GROUP 4 (1000 MG/KG)

21	218	252	289	315	328	---	---	---
22	220	252	283	310	323	---	---	---
23	219	245	276	294	310	---	---	---
24	234	268	299	322	333	---	---	---
25	224	262	287	302	299	---	---	---
26	208	231	257	279	283	280	301	309
27	207	256	287	307	314	307	332	341
28	231	259	283	310	320	314	334	343
29	216	244	279	297	304	296	324	334
30	229	242	275	302	306	299	324	328

FEMALES

	TREATMENT					RECOVERY		
DAYS	1	8	15	22	28	1	8	14
WEEKS	1	2	3	4	4	1	2	2
ANIMAL								

GROUP 1 (CONTROL)

31	161	179	192	192	207	---	---	---
32	161	169	194	203	209	---	---	---
33	160	174	177	194	197	---	---	---
34	165	177	184	194	206	---	---	---
35	169	182	189	205	215	---	---	---
36	151	172	182	190	201	185	200	205
37	168	187	201	208	213	201	221	223
38	163	174	195	208	212	212	212	218
39	164	176	191	201	204	199	205	213
40	164	186	201	209	222	205	223	222

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APPENDIX 2

NOTOX Project 490627

**BODY WEIGHTS (GRAM)
FEMALES**

	TREATMENT					RECOVERY		
DAYS	1	8	15	22	28	1	8	14
WEEKS	1	2	3	4	4	1	2	2
ANIMAL								

GROUP 2 (30 MG/KG)

41	166	179	202	214	214
42	158	172	186	194	196
43	157	171	183	195	201
44	150	166	178	185	189
45	167	179	192	202	204

GROUP 3 (300 MG/KG)

46	162	169	177	191	196
47	160	165	181	192	188
48	155	174	184	190	192
49	164	183	193	203	207
50	172	177	187	200	203

GROUP 4 (1000 MG/KG)

51	159	170	182	190	190	---	---	---
52	157	171	184	196	197	---	---	---
53	159	164	181	187	185	---	---	---
54	170	179	198	210	207	---	---	---
55	147	162	174	175	182	---	---	---
56	156	176	181	189	190	181	197	201
57	155	179	186	196	201	186	204	204
58	170	180	188	209	212	203	217	217
59	168	179	185	186	198	188	197	198
60	174	183	192	207	211	199	216	217

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APPENDIX 2

**BODY WEIGHT GAIN (%)
MALES**

	TREATMENT					RECOVERY		
DAYS	1	8	15	22	28	1	8	14
WEEKS	1	2	3	4	4	1	2	2
ANIMAL								

GROUP 1 (CONTROL)

1	0	12	25	33	37	---	---	---
2	0	12	25	37	43	---	---	---
3	0	16	30	41	43	---	---	---
4	0	16	33	45	53	---	---	---
5	0	17	37	48	55	---	---	---
6	0	12	27	37	43	40	50	51
7	0	16	36	51	56	53	71	78
8	0	15	30	41	47	43	55	59
9	0	13	25	33	36	32	43	45
10	0	13	31	41	46	43	52	58

GROUP 2 (30 MG/KG)

11	0	14	28	36	40			
12	0	13	27	37	44			
13	0	14	31	43	47			
14	0	11	25	34	38			
15	0	13	27	35	42			

GROUP 3 (300 MG/KG)

16	0	14	30	38	47			
17	0	13	30	39	43			
18	0	14	28	36	41			
19	0	12	27	35	38			
20	0	10	24	32	38			

GROUP 4 (1000 MG/KG)

21	0	16	33	44	50	---	---	---
22	0	15	29	41	47	---	---	---
23	0	12	26	34	42	---	---	---
24	0	15	28	38	42	---	---	---
25	0	17	28	35	33	---	---	---
26	0	11	24	34	36	35	45	49
27	0	24	39	48	52	48	60	65
28	0	12	23	34	39	36	45	48
29	0	13	29	38	41	37	50	55
30	0	6	20	32	34	31	41	43

FEMALES

	TREATMENT					RECOVERY		
DAYS	1	8	15	22	28	1	8	14
WEEKS	1	2	3	4	4	1	2	2
ANIMAL								

GROUP 1 (CONTROL)

31	0	11	19	19	29	---	---	---
32	0	5	20	26	30	---	---	---
33	0	9	11	21	23	---	---	---
34	0	7	12	18	25	---	---	---
35	0	8	12	21	27	---	---	---
36	0	14	21	26	33	23	32	36
37	0	11	20	24	27	20	32	33
38	0	7	20	28	30	30	30	34
39	0	7	16	23	24	21	25	30
40	0	13	23	27	35	25	36	35

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APPENDIX 2

**BODY WEIGHT GAIN (%)
FEMALES**

	TREATMENT					RECOVERY		
DAYS	1	8	15	22	28	1	8	14
WEEKS	1	2	3	4	4	1	2	2
ANIMAL								

GROUP 2 (30 MG/KG)

41	0	8	22	29	29
42	0	9	18	23	24
43	0	9	17	24	28
44	0	11	19	23	26
45	0	7	15	21	22

GROUP 3 (300 MG/KG)

46	0	4	9	18	21
47	0	3	13	20	18
48	0	12	19	23	24
49	0	12	18	24	26
50	0	3	9	16	18

GROUP 4 (1000 MG/KG)

51	0	7	14	19	19	---	---	---
52	0	9	17	25	25	---	---	---
53	0	3	14	18	16	---	---	---
54	0	5	16	24	22	---	---	---
55	0	10	18	19	24	---	---	---
56	0	13	16	21	22	16	26	29
57	0	15	20	26	30	20	32	32
58	0	6	11	23	25	19	28	28
59	0	7	10	11	18	12	17	18
60	0	5	10	19	21	14	24	25

APPENDIX 2

**FOOD CONSUMPTION (G/ANIMAL/DAY)
MALES**

	TREATMENT				RECOVERY	
DAYS	1-8	8-15	15-22	22-28	1-8	8-14
WEEKS	1-2	2-3	3-4	4	1-2	2
CAGE						

GROUP 1 (CONTROL)

1	21	23	23	25	---	---
2	21	22	23	24	25	24

GROUP 2 (30 MG/KG)

3	21	22	22	23		
---	----	----	----	----	--	--

GROUP 3 (300 MG/KG)

4	21	23	23	24		
---	----	----	----	----	--	--

GROUP 4 (1000 MG/KG)

5	22	23	24	25	---	---
6	21	22	22	23	24	24

FEMALES

	TREATMENT				RECOVERY	
DAYS	1-8	8-15	15-22	22-28	1-8	8-14
WEEKS	1-2	2-3	3-4	4	1-2	2
CAGE						

GROUP 1 (CONTROL)

7	16	16	17	18	---	---
8	16	16	17	18	20	19

GROUP 2 (30 MG/KG)

9	15	16	16	18		
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GROUP 3 (300 MG/KG)

10	15	15	16	17		
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GROUP 4 (1000 MG/KG)

11	15	16	16	17	---	---
12	16	15	16	17	18	18

APPENDIX 2

**RELATIVE FOOD CONSUMPTION (G/KG BODY WEIGHT/DAY)
MALES**

	TREATMENT				RECOVERY	
DAYS	1-8	8-15	15-22	22-28	1-8	8-14
WEEKS	1-2	2-3	3-4	4	1-2	2
CAGE						

GROUP 1 (CONTROL)

1	85	79	74	78	---	---
2	84	79	74	75	74	70

GROUP 2 (30 MG/KG)

3	84	77	73	73		
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GROUP 3 (300 MG/KG)

4	84	80	74	77		
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GROUP 4 (1000 MG/KG)

5	87	81	77	80	---	---
6	86	81	74	74	75	72

FEMALES

	TREATMENT				RECOVERY	
DAYS	1-8	8-15	15-22	22-28	1-8	8-14
WEEKS	1-2	2-3	3-4	4	1-2	2
CAGE						

GROUP 1 (CONTROL)

7	90	88	88	89	---	---
8	91	84	84	87	92	88

GROUP 2 (30 MG/KG)

9	87	83	81	87		
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GROUP 3 (300 MG/KG)

10	87	83	83	88		
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GROUP 4 (1000 MG/KG)

11	90	85	84	87	---	---
12	88	81	83	86	86	85

APPENDIX 2

HAEMATOTOLOGY
MALES
END OF TREATMENT

ANIMAL	WBC 10E9/L	Neutrophils %WBC	Lymphocytes %WBC	Monocytes %WBC	Eosinophils %WBC
GROUP 1 (CONTROL)					
1	16.4	12.1	85.7	1.0	0.8
2	10.7	11.4	85.7	1.2	1.2
3	9.2	15.0	80.0	4.0	1.0
4	10.1	14.3	83.0	2.0	0.5
5	8.3	15.4	81.2	2.2	0.9
6	13.0	10.6	85.7	2.4	0.6
7	13.0	8.8	88.7	1.3	0.9
8	7.5	20.1	77.6	1.2	0.8
9	9.1	17.6	80.5	1.0	0.7
10	8.6	10.9	86.4	1.4	0.9
GROUP 2 (30 MG/KG)					
11	11.8	10.5	87.2	1.2	0.7
12	10.9	8.6	89.3	1.3	0.3
13	6.6	11.9	86.0	1.2	0.5
14	6.8	10.6	87.4	1.1	0.6
15	15.0	7.0	87.0	4.0	2.0
GROUP 3 (300 MG/KG)					
16	7.5	12.9	84.0	1.7	0.8
17	9.6	12.1	85.9	0.9	0.9
18	13.0	10.0	87.9	1.2	0.7
19	11.0	13.7	82.9	2.2	0.9
20	11.8	14.0	83.0	2.2	0.2
GROUP 4 (1000 MG/KG)					
21	9.7	12.0	86.1	1.0	0.7
22	14.0	15.2	81.9	1.7	0.9
23	10.9	11.3	86.3	1.4	0.7
24	11.8	13.1	84.2	1.9	0.6
25	14.9	13.0	82.0	5.0	0.0
26	11.9	14.8	82.3	1.6	0.7
27	11.3	18.4	79.1	1.7	0.5
28	11.0	14.7	82.5	1.9	0.5
29	11.2	13.8	82.9	1.9	1.1
30	11.6	8.8	89.1	1.1	0.4

MALES
END OF TREATMENT

ANIMAL	Basophils %WBC	Red blood cells 10E12/L	Reticulocytes %RBC	RDW %	Haemoglobin mmol/L
GROUP 1 (CONTROL)					
1	0.4	8.54	3.2	20.6	10.1
2	0.5	8.20	2.7	11.8	9.7
3	0.0	9.02	2.7	11.9	10.0
4	0.4	8.38	3.0	12.1	9.7
5	0.3	8.17	3.7	12.7	9.2
6	0.6	9.48	2.5	11.4	10.5
7	0.3	8.04	2.7	14.7	9.5
8	0.2	8.71	3.4	11.5	9.8
9	0.3	9.49	3.6	12.8	10.1
10	0.4	9.10	3.4	12.2	10.2

APPENDIX 2

**HAEMATOLOGY
MALES
END OF TREATMENT**

ANIMAL	Basophils %WBC	Red blood cells 10E12/L	Reticulocytes %RBC	RDW %	Haemoglobin mmol/L
GROUP 2 (30 MG/KG)					
11	0.4	8.60	2.6	11.5	9.7
12	0.4	8.31	3.0	11.7	9.3
13	0.3	8.30	2.6	12.7	9.2
14	0.3	8.14	2.2	10.8	9.1
15	0.0	8.11	3.6	19.5	9.5
GROUP 3 (300 MG/KG)					
16	0.5	8.02	3.5	12.2	9.1
17	0.2	8.83	2.9	11.8	9.9
18	0.3	8.29	3.5	12.2	9.8
19	0.3	8.85	3.8	12.4	9.6
20	0.6	8.38	3.7	12.4	9.5
GROUP 4 (1000 MG/KG)					
21	0.3	7.74	3.9	12.8	8.8
22	0.4	8.54	4.0	12.7	9.6
23	0.3	8.45	2.9	11.3	9.7
24	0.3	7.62	4.9	14.1	8.9
25	0.0	9.02	2.9	12.3	9.8
26	0.6	8.90	3.3	13.1	9.5
27	0.3	8.15	3.7	12.7	9.0
28	0.3	8.48	3.7	12.4	9.3
29	0.3	8.37	3.7	13.2	9.2
30	0.6	8.36	3.6	12.9	9.6

**MALES
END OF TREATMENT**

ANIMAL	Haematocrit L/L	MCV fL	MCH fmol	MCHC mmol/L	Platelets 10E9/L
GROUP 1 (CONTROL)					
1	0.445	52.1	1.18	22.66	976
2	0.429	52.3	1.18	22.55	988
3	0.466	51.6	1.11	21.51	954
4	0.442	52.8	1.15	21.85	756
5	0.425	52.0	1.13	21.66	811
6	0.492	51.9	1.11	21.41	1157
7	0.424	52.8	1.18	22.28	1098
8	0.454	52.1	1.13	21.66	1018
9	0.482	50.8	1.07	21.02	1088
10	0.465	51.1	1.12	21.92	965
GROUP 2 (30 MG/KG)					
11	0.448	52.1	1.12	21.59	1052
12	0.431	51.8	1.12	21.66	943
13	0.420	50.6	1.11	21.94	1069
14	0.410	50.4	1.12	22.30	1000
15	0.426	52.5	1.17	22.26	938
GROUP 3 (300 MG/KG)					
16	0.417	52.1	1.13	21.72	813
17	0.451	51.1	1.12	21.85	992
18	0.441	53.2	1.19	22.34	1048
19	0.454	51.3	1.09	21.24	1225
20	0.450	53.7	1.13	21.02	991

APPENDIX 2

**HAEMATOLOGY
MALES
END OF TREATMENT**

ANIMAL	Haematocrit L/L	MCV fL	MCH fmol	MCHC mmol/L	Platelets 10E9/L
GROUP 4 (1000 MG/KG)					
21	0.411	53.0	1.14	21.46	868
22	0.447	52.3	1.12	21.45	1114
23	0.441	52.2	1.15	22.08	1034
24	0.401	52.6	1.17	22.15	1236
25	0.455	50.4	1.09	21.51	1172
26	0.448	50.3	1.06	21.11	1039
27	0.416	51.0	1.11	21.73	982
28	0.445	52.4	1.10	21.01	919
29	0.428	51.1	1.10	21.45	947
30	0.444	53.1	1.14	21.56	869

**MALES
END OF TREATMENT**

ANIMAL	PT s	APTT s
GROUP 1 (CONTROL)		
1	16.3	15.5
2	18.5	17.1
3	15.8	13.0
4	14.7	12.4
5	18.3	16.4
6	18.2	19.3
7	19.2	18.4
8	14.5	---
9	17.6	20.1
10	16.6	14.0

GROUP 2 (30 MG/KG)		
11	17.4	19.5
12	17.1	16.1
13	17.6	15.2
14	18.5	16.5
15	16.8	18.1

GROUP 3 (300 MG/KG)		
16	15.8	12.1
17	14.6	12.4
18	15.2	13.3
19	15.5	14.4
20	16.3	20.2

GROUP 4 (1000 MG/KG)		
21	18.6	17.4
22	18.3	16.0
23	15.4	14.5
24	16.6	18.6
25	16.1	17.3
26	17.3	15.5
27	15.7	17.1
28	16.8	16.4
29	17.1	18.6
30	15.9	17.6

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APPENDIX 2

**HAEMATOLOGY
MALES
END OF RECOVERY**

ANIMAL	WBC 10E9/L	Neutrophils %WBC	Lymphocytes %WBC	Monocytes %WBC	Eosinophils %WBC
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GROUP 1 (CONTROL)

6	---	---	---	---	---
7	13.4	11.1	85.0	2.2	1.2
8	7.7	22.6	72.7	2.6	1.6
9	---	---	---	---	---
10	7.5	11.2	84.8	2.2	1.4

GROUP 4 (1000 MG/KG)

26	8.0	26.0	69.9	2.4	1.2
27	10.3	15.9	80.8	2.0	0.8
28	12.1	14.2	81.9	2.9	0.6
29	9.0	17.5	78.5	2.2	1.4
30	10.7	10.4	86.5	1.7	1.0

**MALES
END OF RECOVERY**

ANIMAL	Basophils %WBC	Red blood cells 10E12/L	Reticulocytes %RBC	RDW %	Haemoglobin mmol/L
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GROUP 1 (CONTROL)

6	---	---	---	---	---
7	0.4	7.99	2.6	19.5	9.6
8	0.5	8.44	3.1	12.8	9.6
9	---	---	---	---	---
10	0.4	9.37	2.9	13.1	10.4

GROUP 4 (1000 MG/KG)

26	0.5	8.37	3.2	15.3	9.3
27	0.4	8.37	4.3	14.9	9.5
28	0.4	8.41	3.6	14.3	9.6
29	0.4	8.16	4.2	14.9	9.2
30	0.4	8.25	3.3	14.2	9.8

**MALES
END OF RECOVERY**

ANIMAL	Haematocrit L/L	MCV fL	MCH fmol	MCHC mmol/L	Platelets 10E9/L
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GROUP 1 (CONTROL)

6	---	---	---	---	---
7	0.426	53.3	1.20	22.60	1093
8	0.436	51.6	1.13	21.97	1175
9	---	---	---	---	---
10	0.471	50.3	1.11	22.02	772

GROUP 4 (1000 MG/KG)

26	0.428	51.2	1.11	21.63	942
27	0.441	52.7	1.14	21.55	989
28	0.460	54.7	1.14	20.81	839
29	0.430	52.7	1.12	21.33	964
30	0.452	54.8	1.19	21.70	1046

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APPENDIX 2

**HAEMATOLOGY
MALES
END OF RECOVERY**

ANIMAL	PT s	APTT s
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GROUP 1 (CONTROL)

6	---	---
7	18.7	14.6
8	17.1	19.9
9	16.1	14.3
10	---	---

GROUP 4 (1000 MG/KG)

26	---	---
27	17.3	19.6
28	17.9	16.8
29	17.9	20.6
30	16.7	14.5

**FEMALES
END OF TREATMENT**

ANIMAL	WBC 10E9/L	Neutrophils %WBC	Lymphocytes %WBC	Monocytes %WBC	Eosinophils %WBC
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GROUP 1 (CONTROL)

31	3.5	15.7	81.0	1.7	1.4
32	5.5	11.2	86.1	1.0	1.5
33	8.4	12.6	83.8	1.5	1.7
34	6.3	10.0	87.5	1.2	1.0
35	3.3	11.8	84.5	1.4	2.1
36	4.0	19.3	76.2	1.6	2.7
37	5.8	16.6	79.7	1.5	1.6
38	6.0	11.8	84.9	1.8	1.2
39	6.9	7.4	90.8	1.1	0.5
40	4.9	17.7	78.6	1.8	1.5

GROUP 2 (30 MG/KG)

41	7.1	10.3	87.5	1.3	0.7
42	6.3	14.1	82.9	2.0	0.8
43	3.6	14.5	84.2	0.7	0.4
44	4.6	16.3	80.8	1.8	1.0
45	3.0	14.5	83.4	0.9	1.0

GROUP 3 (300 MG/KG)

46	6.1	5.0	93.0	2.0	0.0
47	7.2	10.1	87.6	1.6	0.5
48	8.5	17.5	79.7	1.6	0.6
49	4.5	18.2	80.0	1.0	0.6
50	6.8	14.0	81.4	3.4	0.8

GROUP 4 (1000 MG/KG)

51	8.3	6.3	92.2	0.8	0.4
52	8.2	15.6	81.4	1.4	1.1
53	5.5	10.7	87.9	0.9	0.5
54	13.1	9.4	88.5	1.2	0.7
55	3.8	13.7	85.1	0.8	0.1
56	7.0	14.3	82.4	1.8	1.3
57	5.0	13.2	84.9	1.1	0.4
58	6.5	11.5	85.8	1.3	1.1
59	7.9	8.2	89.2	1.9	0.4
60	8.3	7.0	91.3	1.2	0.3

APPENDIX 2

**HAEMATOLOGY
FEMALES
END OF TREATMENT**

ANIMAL	Basophils %WBC	Red blood cells 10E12/L	Reticulocytes %RBC	RDW %	Haemoglobin mmol/L
GROUP 1 (CONTROL)					
31	0.1	7.49	3.9	11.3	8.7
32	0.3	8.05	3.7	12.4	9.0
33	0.3	8.02	3.0	12.5	9.4
34	0.3	8.46	3.0	11.1	9.4
35	0.3	7.97	3.4	11.6	9.3
36	0.2	7.41	3.9	11.7	8.2
37	0.6	7.80	3.5	11.8	9.0
38	0.2	8.60	2.2	10.8	9.5
39	0.2	8.28	3.4	11.0	9.1
40	0.3	7.96	3.6	11.2	9.1
GROUP 2 (30 MG/KG)					
41	0.2	8.09	3.5	11.8	9.2
42	0.2	7.93	3.9	11.6	9.0
43	0.2	7.66	4.0	12.3	8.5
44	0.2	7.36	4.4	12.3	8.5
45	0.1	8.12	3.0	10.9	9.0
GROUP 3 (300 MG/KG)					
46	0.0	7.84	3.7	11.8	8.9
47	0.2	8.65	2.6	10.9	9.6
48	0.6	8.74	3.9	12.6	10.0
49	0.2	8.33	4.8	12.5	9.0
50	0.3	8.19	4.2	11.7	9.5
GROUP 4 (1000 MG/KG)					
51	0.3	8.03	2.5	11.4	8.9
52	0.4	8.33	3.8	12.0	9.2
53	0.1	8.05	2.5	10.6	8.9
54	0.2	8.87	2.7	10.9	9.7
55	0.3	7.29	4.8	12.5	8.2
56	0.2	8.03	3.6	12.1	8.7
57	0.3	7.69	4.3	12.0	8.8
58	0.3	8.05	3.8	11.6	9.1
59	0.3	7.89	3.3	11.5	8.5
60	0.2	8.34	3.1	11.5	8.9

**FEMALES
END OF TREATMENT**

ANIMAL	Haematocrit L/L	MCV fL	MCH fmol	MCHC mmol/L	Platelets 10E9/L
GROUP 1 (CONTROL)					
31	0.411	54.9	1.16	21.16	1040
32	0.428	53.2	1.12	21.04	885
33	0.432	53.8	1.17	21.67	1011
34	0.444	52.5	1.11	21.10	1186
35	0.427	53.6	1.17	21.75	1319
36	0.376	50.7	1.11	21.90	1057
37	0.410	52.7	1.16	22.04	1081
38	0.436	50.6	1.11	21.91	1103
39	0.427	51.6	1.10	21.38	1153
40	0.410	51.5	1.15	22.24	1220

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APPENDIX 2

**HAEMATOLOGY
FEMALES
END OF TREATMENT**

ANIMAL	Haematocrit L/L	MCV fL	MCH fmol	MCHC mmol/L	Platelets 10E9/L
GROUP 2 (30 MG/KG)					
41	0.422	52.1	1.14	21.79	1064
42	0.407	51.4	1.13	22.06	864
43	0.390	51.0	1.11	21.85	870
44	0.369	50.1	1.15	23.05	830
45	0.398	49.0	1.11	22.57	716
GROUP 3 (300 MG/KG)					
46	0.390	49.8	1.14	22.87	805
47	0.443	51.2	1.11	21.65	912
48	0.449	51.3	1.14	22.22	993
49	0.425	51.0	1.08	21.21	1032
50	0.446	54.5	1.16	21.37	987
GROUP 4 (1000 MG/KG)					
51	0.406	50.5	1.11	22.05	921
52	0.433	52.0	1.10	21.22	1043
53	0.404	50.1	1.11	22.07	769
54	0.450	50.7	1.09	21.55	1033
55	0.362	49.6	1.12	22.63	707
56	0.397	49.4	1.08	21.91	830
57	0.409	53.3	1.14	21.38	844
58	0.423	52.6	1.13	21.47	987
59	0.380	48.2	1.08	22.44	770
60	0.412	49.4	1.07	21.66	1095

**FEMALES
END OF TREATMENT**

ANIMAL	PT s	APTT s
GROUP 1 (CONTROL)		
31	16.9	15.7
32	17.2	17.7
33	16.1	16.8
34	17.0	16.4
35	17.2	15.5
36	15.9	14.0
37	16.7	16.3
38	16.4	19.6
39	17.2	18.6
40	17.2	19.7
GROUP 2 (30 MG/KG)		
41	16.7	17.3
42	15.8	15.4
43	16.3	14.8
44	16.1	15.8
45	17.9	13.3
GROUP 3 (300 MG/KG)		
46	15.4	12.8
47	17.2	19.9
48	16.6	19.7
49	17.7	17.3
50	14.9	17.0

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APPENDIX 2

**HAEMATOLOGY
FEMALES
END OF TREATMENT**

ANIMAL	PT s	APTT s
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GROUP 4 (1000 MG/KG)

51	16.2	16.8
52	16.1	18.2
53	15.5	14.4
54	17.0	20.1
55	16.2	14.0
56	17.2	20.2
57	17.0	15.8
58	17.5	20.0
59	16.7	17.6
60	16.3	16.3

**FEMALES
END OF RECOVERY**

ANIMAL	WBC 10E9/L	Neutrophils %WBC	Lymphocytes %WBC	Monocytes %WBC	Eosinophils %WBC
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GROUP 1 (CONTROL)

36	4.4	15.4	79.6	2.7	2.1
37	7.4	11.1	84.0	2.0	2.1
38	2.7	21.2	73.6	3.3	1.4
39	5.5	6.1	91.4	1.1	0.9
40	5.0	8.4	88.3	1.2	1.7

GROUP 4 (1000 MG/KG)

56	4.3	15.2	80.7	2.0	1.9
57	6.3	7.7	89.4	1.7	0.9
58	3.9	14.7	81.1	1.7	2.4
59	3.5	11.5	86.6	1.4	0.3
60	7.9	10.6	86.3	2.1	0.7

**FEMALES
END OF RECOVERY**

ANIMAL	Basophils %WBC	Red blood cells 10E12/L	Reticulocytes %RBC	RDW %	Haemoglobin mmol/L
--------	-------------------	----------------------------	-----------------------	----------	-----------------------

GROUP 1 (CONTROL)

36	0.3	7.99	2.5	12.2	9.3
37	0.7	8.64	2.4	12.7	10.0
38	0.5	7.64	2.2	12.3	8.9
39	0.5	8.44	2.7	12.6	9.7
40	0.4	8.31	2.2	12.7	9.7

GROUP 4 (1000 MG/KG)

56	0.3	7.43	2.1	14.4	8.5
57	0.3	8.67	2.4	13.4	10.3
58	0.2	7.55	3.5	13.5	9.1
59	0.2	6.91	3.1	15.2	8.0
60	0.3	8.15	3.4	15.0	9.2

APPENDIX 2

**HAEMATOLOGY
FEMALES
END OF RECOVERY**

ANIMAL	Haematocrit L/L	MCV fL	MCH fmol	MCHC mmol/L	Platelets 10E9/L
GROUP 1 (CONTROL)					
36	0.410	51.3	1.16	22.61	1116
37	0.461	53.3	1.16	21.79	1147
38	0.396	51.9	1.17	22.48	1031
39	0.442	52.4	1.15	21.91	1034
40	0.439	52.8	1.17	22.10	1287

GROUP 4 (1000 MG/KG)					
56	0.376	50.6	1.14	22.47	835
57	0.472	54.5	1.18	21.75	859
58	0.412	54.5	1.20	22.03	962
59	0.354	51.2	1.15	22.49	697
60	0.429	52.6	1.13	21.55	1031

**FEMALES
END OF RECOVERY**

ANIMAL	PT s	APTT s
GROUP 1 (CONTROL)		
36	17.6	17.2
37	18.2	19.7
38	16.2	15.0
39	19.0	20.7
40	18.2	17.0

GROUP 4 (1000 MG/KG)		
56	---	---
57	15.5	14.9
58	17.3	19.4
59	15.8	14.8
60	17.6	18.3

APPENDIX 2

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**CLINICAL BIOCHEMISTRY
MALES
END OF TREATMENT**

ANIMAL	ALAT U/L	ASAT U/L	ALP U/L	Total protein g/L	Albumin g/L
GROUP 1 (CONTROL)					
1	39.6	66.0	185	64.8	33.0
2	61.1	80.4	220	61.7	30.3
3	53.4	77.9	121	69.0	32.9
4	42.1	60.8	121	63.8	31.8
5	55.2	84.0	148	60.9	31.1
6	52.4	82.2	211	67.4	34.9
7	46.2	60.4	187	66.9	32.6
8	39.9	84.9	114	64.5	32.2
9	46.0	79.8	151	66.0	34.1
10	77.3	70.1	266	63.6	32.5
GROUP 2 (30 MG/KG)					
11	32.2	66.4	94	63.3	31.6
12	30.8	78.5	167	60.1	31.0
13	38.9	72.7	162	66.7	33.0
14	47.9	76.6	117	60.0	30.7
15	44.0	77.1	162	61.6	31.3
GROUP 3 (300 MG/KG)					
16	37.2	73.7	149	63.4	31.3
17	61.3	79.8	197	66.4	33.1
18	29.9	73.8	124	65.3	31.9
19	66.6	78.1	232	66.9	33.2
20	29.5	77.7	165	66.1	32.3
GROUP 4 (1000 MG/KG)					
21	38.5	78.4	119	62.4	30.9
22	44.4	75.7	135	63.5	31.0
23	50.4	91.8	221	65.5	32.6
24	34.9	63.1	101	66.6	33.1
25	33.5	81.3	125	68.6	34.5
26	49.1	74.5	172	64.4	32.7
27	32.9	75.1	125	61.5	31.1
28	44.0	93.2	154	64.9	31.6
29	30.2	70.8	153	65.6	32.5
30	41.5	70.3	115	68.6	34.3

**MALES
END OF TREATMENT**

ANIMAL	Total bilirubin umol/L	Urea mmol/L	Creatinine umol/L	Glucose mmol/L	Cholesterol mmol/L
GROUP 1 (CONTROL)					
1	2.8	8.6	41.2	8.22	2.12
2	2.5	9.8	42.6	5.66	2.11
3	2.8	9.8	41.9	10.15	2.07
4	2.4	6.5	41.2	6.67	2.54
5	2.3	7.4	39.8	8.37	1.91
6	2.6	7.3	38.4	7.54	1.30
7	2.1	9.5	40.5	11.00	2.10
8	1.8	7.7	41.9	8.03	2.72
9	2.1	7.9	41.9	11.60	1.28
10	1.9	8.2	39.8	10.18	1.69

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**CLINICAL BIOCHEMISTRY
MALES
END OF TREATMENT**

ANIMAL	Total bilirubin umol/L	Urea mmol/L	Creatinine umol/L	Glucose mmol/L	Cholesterol mmol/L
GROUP 2 (30 MG/KG)					
11	2.0	7.8	43.3	10.76	1.98
12	2.1	7.4	41.9	7.27	1.69
13	1.8	8.4	38.4	8.46	2.13
14	1.7	10.7	39.1	8.98	2.02
15	2.1	6.2	37.7	8.54	2.35
GROUP 3 (300 MG/KG)					
16	1.8	6.6	41.2	6.56	2.71
17	1.7	10.0	41.9	11.86	2.65
18	1.8	7.4	38.4	9.22	1.91
19	1.8	9.7	43.3	11.85	2.80
20	1.6	6.2	37.7	5.98	1.81
GROUP 4 (1000 MG/KG)					
21	1.8	10.5	46.1	6.57	2.32
22	1.9	7.5	39.1	9.78	2.24
23	1.5	7.9	41.2	9.33	1.99
24	2.0	9.0	41.9	10.03	1.72
25	1.8	9.6	45.4	13.25	1.85
26	2.0	9.8	43.3	12.30	1.74
27	1.6	5.7	39.1	6.96	2.56
28	2.0	7.9	41.2	7.12	1.64
29	2.3	8.7	43.3	7.63	2.25
30	1.8	9.6	39.8	11.63	1.44

**MALES
END OF TREATMENT**

ANIMAL	Bile Acids umol/l	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Inorg.Phos mmol/L
GROUP 1 (CONTROL)						
1	48.7	141.6	4.14	104	2.87	1.99
2	70.2	142.3	4.24	105	2.81	2.29
3	39.8	140.5	4.45	103	2.85	2.11
4	54.2	142.7	3.86	104	2.82	2.20
5	44.5	141.1	4.22	103	2.84	2.21
6	43.6	142.0	4.14	106	2.82	1.88
7	41.8	141.5	4.33	103	2.92	2.34
8	27.8	141.3	4.12	103	2.77	2.32
9	35.1	141.2	3.90	103	2.81	2.10
10	34.2	141.8	4.14	105	2.80	2.08
GROUP 2 (30 MG/KG)						
11	45.1	139.1	3.75	103	2.75	2.02
12	36.6	142.6	4.11	104	2.72	2.21
13	70.8	141.4	4.47	104	2.89	2.03
14	37.3	141.4	3.62	104	2.81	2.18
15	63.8	140.1	4.06	101	2.82	2.40
GROUP 3 (300 MG/KG)						
16	26.8	142.0	4.22	105	2.81	2.10
17	40.3	142.1	4.57	104	2.90	2.36
18	22.1	139.4	4.54	104	2.82	2.26
19	22.0	141.1	4.30	103	2.83	2.07
20	15.3	143.6	4.05	106	2.76	2.11

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**CLINICAL BIOCHEMISTRY
MALES
END OF TREATMENT**

ANIMAL	Bile Acids umol/l	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Inorg.Phos mmol/L
GROUP 4 (1000 MG/KG)						
21	22.8	142.1	3.95	106	2.80	2.39
22	35.9	141.1	4.24	104	2.87	2.22
23	26.6	141.7	4.15	106	2.75	2.26
24	41.1	142.6	4.19	105	2.90	2.23
25	29.4	140.0	4.54	103	2.87	2.06
26	32.7	141.2	4.28	102	2.82	2.07
27	25.9	143.2	3.70	104	2.79	2.22
28	17.3	140.4	4.13	105	2.81	2.17
29	34.1	140.7	3.91	103	2.86	2.15
30	27.8	142.2	4.36	104	2.96	2.10

**MALES
END OF RECOVERY**

ANIMAL	ALAT U/L	ASAT U/L	ALP U/L	Total protein g/L	Albumin g/L
GROUP 1 (CONTROL)					
6	45.9	86.2	173	66.0	34.0
7	41.6	75.9	183	67.0	31.9
8	30.2	68.2	113	63.8	32.5
9	40.6	79.7	138	63.5	32.8
10	54.0	65.7	200	62.1	31.4
GROUP 4 (1000 MG/KG)					
26	59.3	94.1	150	63.0	32.5
27	31.8	72.1	140	62.9	32.6
28	49.7	107.6	150	62.2	31.4
29	27.8	64.1	136	63.9	32.6
30	60.0	64.2	107	66.8	33.8

**MALES
END OF RECOVERY**

ANIMAL	Total bilirubin umol/L	Urea mmol/L	Creatinine umol/L	Glucose mmol/L	Cholesterol mmol/L
GROUP 1 (CONTROL)					
6	2.7	6.9	41.9	10.44	1.52
7	2.3	7.6	42.6	12.80	2.26
8	2.4	6.1	41.9	9.85	2.37
9	2.1	7.8	44.1	12.01	1.45
10	2.0	7.7	41.1	10.44	1.69
GROUP 4 (1000 MG/KG)					
26	2.0	10.0	44.9	12.77	1.75
27	2.3	5.5	41.9	9.83	1.87
28	2.4	8.3	42.6	10.08	1.26
29	1.9	7.6	41.1	8.95	2.07
30	2.3	10.7	42.6	17.97	1.58

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**CLINICAL BIOCHEMISTRY
MALES
END OF RECOVERY**

ANIMAL	Bile Acids umol/l	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Inorg.Phos mmol/L
GROUP 1 (CONTROL)						
6	25.9	141.2	4.35	104	2.78	1.76
7	25.3	141.4	4.16	103	2.92	2.00
8	21.2	141.9	4.06	102	2.86	2.06
9	40.7	142.2	3.52	102	2.78	1.94
10	29.9	142.3	3.94	104	2.83	2.07
GROUP 4 (1000 MG/KG)						
26	27.9	139.8	4.41	103	2.81	2.22
27	46.4	142.4	4.22	104	2.85	2.27
28	14.4	142.6	4.60	106	2.91	2.45
29	25.7	141.4	4.13	105	2.91	2.37
30	26.4	140.6	4.83	102	2.97	2.09

**FEMALES
END OF TREATMENT**

ANIMAL	ALAT U/L	ASAT U/L	ALP U/L	Total protein g/L	Albumin g/L
GROUP 1 (CONTROL)					
31	32.1	72.1	90	65.5	33.2
32	35.2	80.5	65	62.5	33.3
33	42.0	58.6	96	66.8	34.9
34	60.1	72.0	61	70.0	36.7
35	27.0	67.2	59	63.9	32.7
36	31.7	76.0	72	63.8	33.3
37	50.6	70.8	77	61.6	31.9
38	35.8	65.9	52	68.5	36.3
39	49.9	82.2	98	68.0	35.9
40	32.9	69.5	94	68.7	35.5
GROUP 2 (30 MG/KG)					
41	66.1	96.8	116	66.7	33.7
42	36.0	71.2	71	65.1	33.5
43	28.7	68.8	48	68.4	35.7
44	33.9	84.3	66	62.9	32.5
45	49.9	72.8	49	69.2	34.1
GROUP 3 (300 MG/KG)					
46	49.8	82.3	52	67.9	35.9
47	34.8	64.4	55	67.2	35.3
48	32.3	83.5	62	68.3	35.4
49	27.0	71.2	53	68.8	35.4
50	38.0	76.3	57	71.1	36.8
GROUP 4 (1000 MG/KG)					
51	48.5	81.9	53	67.5	34.9
52	22.6	71.1	68	71.0	36.6
53	36.0	70.2	72	69.7	35.4
54	44.5	81.2	81	71.2	37.3
55	29.1	99.3	60	60.7	31.5
56	23.8	66.5	59	67.2	34.5
57	19.8	88.9	67	66.8	34.0
58	27.7	74.4	51	65.1	33.8
59	43.6	76.2	70	63.5	32.4
60	46.8	86.2	70	69.9	36.5

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**CLINICAL BIOCHEMISTRY
FEMALES
END OF TREATMENT**

ANIMAL	Total bilirubin umol/L	Urea mmol/L	Creatinine umol/L	Glucose mmol/L	Cholesterol mmol/L
GROUP 1 (CONTROL)					
31	2.1	5.1	41.2	8.57	1.23
32	2.1	7.7	43.3	6.75	1.28
33	2.2	10.7	41.2	7.55	2.10
34	1.9	10.2	42.6	8.59	1.71
35	1.8	8.7	45.4	7.16	1.61
36	2.1	8.5	44.7	7.30	1.41
37	2.1	9.3	46.8	8.53	1.34
38	2.6	7.8	43.3	6.95	2.20
39	2.3	12.5	46.1	7.02	1.25
40	2.0	8.7	44.0	7.21	1.79
GROUP 2 (30 MG/KG)					
41	3.4	8.4	43.3	9.05	2.67
42	2.5	9.7	44.7	6.88	1.79
43	2.3	9.2	45.4	5.88	1.98
44	1.9	8.8	44.0	7.58	1.86
45	1.8	10.5	46.1	7.15	1.59
GROUP 3 (300 MG/KG)					
46	2.3	9.8	42.6	5.86	2.53
47	2.3	8.3	43.3	6.69	2.31
48	2.1	8.5	43.3	6.55	1.97
49	1.7	7.2	41.9	5.78	2.05
50	2.1	9.0	41.9	7.28	2.50
GROUP 4 (1000 MG/KG)					
51	2.3	9.5	40.5	5.97	3.32
52	2.6	8.2	42.6	7.25	2.21
53	2.6	8.6	41.2	6.87	2.99
54	2.1	9.9	44.0	9.13	2.16
55	2.1	11.9	45.4	6.57	1.91
56	2.1	8.0	44.0	6.13	1.88
57	1.6	8.1	46.8	7.61	2.21
58	2.1	12.3	48.2	6.75	2.12
59	2.0	8.5	37.7	7.07	2.90
60	2.4	8.6	42.6	6.01	1.54

**FEMALES
END OF TREATMENT**

ANIMAL	Bile Acids umol/l	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Inorg.Phos mmol/L
GROUP 1 (CONTROL)						
31	16.1	138.9	3.53	104	2.69	1.67
32	25.1	140.9	3.37	106	2.69	1.75
33	34.8	140.6	3.54	106	2.88	2.14
34	40.0	140.1	3.64	104	2.87	1.87
35	20.0	143.1	3.66	107	2.80	2.22
36	9.2	141.9	3.19	107	2.63	1.61
37	24.7	140.1	3.40	106	2.69	1.48
38	22.0	140.2	3.74	105	2.92	1.85
39	25.0	144.0	3.97	108	2.83	2.49
40	19.3	141.4	3.41	106	2.79	1.97

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**CLINICAL BIOCHEMISTRY
FEMALES
END OF TREATMENT**

ANIMAL	Bile Acids umol/l	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Inorg.Phos mmol/L
GROUP 2 (30 MG/KG)						
41	56.7	140.0	3.82	103	2.86	1.74
42	33.9	140.7	3.30	104	2.72	1.70
43	33.0	139.9	3.39	104	2.74	1.68
44	20.0	141.2	3.20	108	2.59	1.55
45	43.0	140.3	3.67	103	2.77	1.82
GROUP 3 (300 MG/KG)						
46	48.5	141.9	3.88	105	2.90	1.83
47	14.5	139.7	3.72	104	2.76	1.54
48	26.8	140.5	3.39	104	2.74	1.81
49	28.3	141.5	3.37	107	2.64	1.98
50	18.0	141.9	3.82	106	2.97	1.93
GROUP 4 (1000 MG/KG)						
51	41.0	141.2	3.81	105	2.90	2.10
52	15.0	140.0	3.71	106	2.81	1.54
53	38.0	141.0	3.81	106	2.91	1.67
54	22.9	138.2	3.84	103	2.91	1.78
55	20.3	141.5	3.74	107	2.55	2.15
56	24.7	139.7	3.58	105	2.77	1.40
57	32.6	139.7	3.20	103	2.62	1.83
58	18.9	141.7	3.75	108	2.74	1.96
59	32.6	142.2	3.93	106	2.78	1.82
60	43.4	140.7	3.73	105	2.81	1.80

**FEMALES
END OF RECOVERY**

ANIMAL	ALAT U/L	ASAT U/L	ALP U/L	Total protein g/L	Albumin g/L
GROUP 1 (CONTROL)					
36	36.6	77.5	59	65.0	32.5
37	42.2	73.9	65	64.1	33.4
38	27.0	62.9	47	69.4	36.1
39	43.2	78.4	98	65.1	33.7
40	45.4	73.6	86	68.1	34.4
GROUP 4 (1000 MG/KG)					
56	32.0	128.8	57	67.0	36.1
57	26.2	75.2	56	67.0	34.5
58	27.7	80.2	48	64.7	34.4
59	48.8	72.7	66	60.5	32.7
60	33.1	78.5	77	67.1	35.8

**FEMALES
END OF RECOVERY**

ANIMAL	Total bilirubin umol/L	Urea mmol/L	Creatinine umol/L	Glucose mmol/L	Cholesterol mmol/L
GROUP 1 (CONTROL)					
36	2.0	8.4	44.1	7.45	1.67
37	2.5	8.0	47.1	8.95	1.53
38	2.4	5.7	41.9	10.71	2.25
39	2.3	7.3	42.6	9.26	1.22
40	2.0	7.9	44.1	8.26	1.84

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APPENDIX 2

**CLINICAL BIOCHEMISTRY
FEMALES
END OF RECOVERY**

ANIMAL	Total bilirubin umol/L	Urea mmol/L	Creatinine umol/L	Glucose mmol/L	Cholesterol mmol/L
GROUP 4 (1000 MG/KG)					
56	2.7	7.0	50.1	6.27	1.22
57	2.4	7.7	50.8	7.19	1.43
58	2.2	10.5	50.8	7.69	1.80
59	2.0	8.3	44.9	9.46	2.14
60	2.3	9.5	46.3	10.47	1.31

**FEMALES
END OF RECOVERY**

ANIMAL	Bile Acids umol/l	Sodium mmol/L	Potassium mmol/L	Chloride mmol/L	Calcium mmol/L	Inorg.Phos mmol/L
GROUP 1 (CONTROL)						
36	15.8	140.9	3.30	105	2.74	1.61
37	31.5	141.4	3.36	105	2.80	1.41
38	25.4	139.7	3.45	103	2.82	1.28
39	15.9	141.1	3.37	103	2.73	1.79
40	15.5	140.7	3.36	104	2.84	1.85
GROUP 4 (1000 MG/KG)						
56	16.4	139.5	3.85	104	2.74	1.47
57	13.7	141.7	3.62	104	2.78	1.92
58	9.1	141.5	3.40	107	2.71	1.80
59	11.3	140.3	3.75	105	2.76	1.72
60	18.6	139.9	3.46	102	2.74	1.78

APPENDIX 2

**MACROSCOPIC FINDINGS
MALES
ALL NECROPSIES**

ANIMAL ORGAN	FINDING	DAY OF DEATH
GROUP 1 (CONTROL)		
1	No findings noted	Scheduled sacrifice, 10Apr2009
2	Seminal vesicles	Scheduled sacrifice, 10Apr2009
3	Right side: reduced in size.	Scheduled sacrifice, 10Apr2009
4	No findings noted	Scheduled sacrifice, 10Apr2009
5	No findings noted	Scheduled sacrifice, 10Apr2009
6	No findings noted	Scheduled sacrifice, 24Apr2009
7	No findings noted	Scheduled sacrifice, 24Apr2009
8	No findings noted	Scheduled sacrifice, 24Apr2009
9	No findings noted	Scheduled sacrifice, 24Apr2009
10	No findings noted	Scheduled sacrifice, 24Apr2009
GROUP 2 (30 MG/KG)		
11	No findings noted	Scheduled sacrifice, 10Apr2009
12	No findings noted	Scheduled sacrifice, 10Apr2009
13	No findings noted	Scheduled sacrifice, 10Apr2009
14	No findings noted	Scheduled sacrifice, 10Apr2009
15	No findings noted	Scheduled sacrifice, 10Apr2009
GROUP 3 (300 MG/KG)		
16	No findings noted	Scheduled sacrifice, 10Apr2009
17	No findings noted	Scheduled sacrifice, 10Apr2009
18	No findings noted	Scheduled sacrifice, 10Apr2009
19	No findings noted	Scheduled sacrifice, 10Apr2009
20	No findings noted	Scheduled sacrifice, 10Apr2009
GROUP 4 (1000 MG/KG)		
21	No findings noted	Scheduled sacrifice, 10Apr2009
22	No findings noted	Scheduled sacrifice, 10Apr2009
23	No findings noted	Scheduled sacrifice, 10Apr2009
24	No findings noted	Scheduled sacrifice, 10Apr2009
25	No findings noted	Scheduled sacrifice, 10Apr2009
26	No findings noted	Scheduled sacrifice, 24Apr2009
27	No findings noted	Scheduled sacrifice, 24Apr2009
28	No findings noted	Scheduled sacrifice, 24Apr2009
29	No findings noted	Scheduled sacrifice, 24Apr2009
30	No findings noted	Scheduled sacrifice, 24Apr2009

**FEMALES
ALL NECROPSIES**

ANIMAL ORGAN	FINDING	DAY OF DEATH
GROUP 1 (CONTROL)		
31	Uterus	Scheduled sacrifice, 10Apr2009
32	Contains fluid.	Scheduled sacrifice, 10Apr2009
33	No findings noted	Scheduled sacrifice, 10Apr2009
34	No findings noted	Scheduled sacrifice, 10Apr2009
35	No findings noted	Scheduled sacrifice, 10Apr2009
36	Clitoral glands	Scheduled sacrifice, 24Apr2009
37	Left side: discolouration, dark red.	Scheduled sacrifice, 24Apr2009
38	No findings noted	Scheduled sacrifice, 24Apr2009
39	Uterus	Scheduled sacrifice, 24Apr2009
40	Clitoral glands	Scheduled sacrifice, 24Apr2009
	Right side: focus/foci, isolated, tan.	Scheduled sacrifice, 24Apr2009
	No findings noted	Scheduled sacrifice, 24Apr2009
GROUP 2 (30 MG/KG)		
41	No findings noted	Scheduled sacrifice, 10Apr2009
42	No findings noted	Scheduled sacrifice, 10Apr2009
43	No findings noted	Scheduled sacrifice, 10Apr2009
44	No findings noted	Scheduled sacrifice, 10Apr2009
45	No findings noted	Scheduled sacrifice, 10Apr2009

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APPENDIX 2

**MACROSCOPIC FINDINGS
FEMALES
ALL NECROPSIES**

ANIMAL ORGAN	FINDING	DAY OF DEATH
GROUP 3 (300 MG/KG)		
46	No findings noted	Scheduled sacrifice, 10Apr2009
47	No findings noted	Scheduled sacrifice, 10Apr2009
48	No findings noted	Scheduled sacrifice, 10Apr2009
49 Uterus	Contains fluid.	Scheduled sacrifice, 10Apr2009
50	No findings noted	Scheduled sacrifice, 10Apr2009
GROUP 4 (1000 MG/KG)		
51	No findings noted	Scheduled sacrifice, 10Apr2009
52	No findings noted	Scheduled sacrifice, 10Apr2009
53	No findings noted	Scheduled sacrifice, 10Apr2009
54	No findings noted	Scheduled sacrifice, 10Apr2009
55 Uterus	Contains fluid.	Scheduled sacrifice, 10Apr2009
56	No findings noted	Scheduled sacrifice, 24Apr2009
57	No findings noted	Scheduled sacrifice, 24Apr2009
58 Uterus	Contains fluid.	Scheduled sacrifice, 24Apr2009
59	No findings noted	Scheduled sacrifice, 24Apr2009
60 Uterus	Contains fluid.	Scheduled sacrifice, 24Apr2009

APPENDIX 2

ORGAN WEIGHTS (GRAM)
MALES
END OF TREATMENT

ANIMAL	BODY W. (GRAM)	BRAIN (GRAM)	HEART (GRAM)	LIVER (GRAM)	THYROID (GRAM)
GROUP 1 (CONTROL)					
1	269	1.87	0.767	7.08	0.020
2	306	2.03	0.850	7.88	0.029
3	298	1.92	0.865	8.08	0.031
4	319	1.96	0.923	8.35	0.019
5	340	2.02	0.918	9.59	0.024
GROUP 2 (30 MG/KG)					
11	299	1.97	0.896	8.44	0.025
12	282	1.88	1.011	7.59	0.042
13	295	1.93	0.868	8.19	0.024
14	283	2.00	0.875	7.47	0.027
15	295	1.82	0.817	8.53	0.030
GROUP 3 (300 MG/KG)					
16	310	1.94	0.878	9.30	0.032
17	296	1.95	0.891	8.80	0.033
18	293	2.07	0.927	8.78	0.024
19	279	1.93	0.831	8.64	0.020
20	284	1.98	0.919	8.75	0.032
GROUP 4 (1000 MG/KG)					
21	303	1.96	0.940	9.03	0.025
22	302	1.76	0.889	9.36	0.036
23	282	1.99	0.757	8.20	0.028
24	311	1.84	0.940	10.07	0.042
25	282	1.90	0.867	8.74	0.030

MALES
END OF TREATMENT

ANIMAL	THYMUS (GRAM)	KIDNEYS (GRAM)	ADRENALS (GRAM)	SPLEEN (GRAM)	TESTES (GRAM)
GROUP 1 (CONTROL)					
1	0.426	1.94	0.058	0.505	2.82
2	0.406	2.13	0.051	0.687	3.10
3	0.366	2.15	0.054	0.514	2.67
4	0.481	2.29	0.059	0.628	3.49
5	0.516	2.63	0.050	0.659	3.28
GROUP 2 (30 MG/KG)					
11	0.400	2.18	0.056	0.490	3.32
12	0.524	2.07	0.064	0.687	2.85
13	0.355	2.37	0.076	0.560	3.56
14	0.374	2.05	0.056	0.599	3.88
15	0.372	2.23	0.073	0.498	3.17
GROUP 3 (300 MG/KG)					
16	0.411	2.20	0.058	0.672	3.19
17	0.328	2.20	0.055	0.561	3.28
18	0.436	2.36	0.068	0.617	3.26
19	0.330	2.13	0.062	0.630	3.18
20	0.387	2.64	0.059	0.657	3.23

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APPENDIX 2

ORGAN WEIGHTS (GRAM)**MALES****END OF TREATMENT**

ANIMAL	THYMUS (GRAM)	KIDNEYS (GRAM)	ADRENALS (GRAM)	SPLEEN (GRAM)	TESTES (GRAM)
GROUP 4 (1000 MG/KG)					
21	0.354	2.15	0.054	0.693	3.27
22	0.557	2.52	0.070	0.671	3.47
23	0.302	2.27	0.060	0.593	3.04
24	0.409	2.60	0.069	0.613	3.38
25	0.264	2.15	0.082	0.577	2.82

MALES**END OF TREATMENT**

ANIMAL	PROSTATE (GRAM)	EPIDIDYMIDES (GRAM)	SEMINAL VES (GRAM)
GROUP 1 (CONTROL)			
1	0.377	0.836	0.921
2	0.432	0.842	0.676
3	0.357	0.833	0.736
4	0.624	0.989	1.085
5	0.420	0.857	1.068
GROUP 2 (30 MG/KG)			
11	0.598	0.836	0.959
12	0.398	0.851	0.718
13	0.589	0.844	0.954
14	0.453	0.986	0.783
15	0.386	0.976	0.825
GROUP 3 (300 MG/KG)			
16	0.378	0.924	1.031
17	0.470	0.870	1.013
18	0.648	0.862	0.772
19	0.576	0.808	0.828
20	0.507	0.944	1.154
GROUP 4 (1000 MG/KG)			
21	0.368	0.960	1.156
22	0.499	0.949	0.858
23	0.441	0.880	1.194
24	0.474	0.973	1.148
25	0.533	0.906	1.183

MALES**END OF RECOVERY**

ANIMAL	BODY W. (GRAM)	BRAIN (GRAM)	HEART (GRAM)	LIVER (GRAM)	THYROIDS (GRAM)
GROUP 1 (CONTROL)					
6	313	1.87	0.885	7.44	0.032
7	396	2.02	1.090	9.62	0.027
8	327	1.91	0.989	8.59	0.024
9	297	1.96	0.934	7.12	0.026
10	329	2.02	0.917	8.91	0.032
GROUP 4 (1000 MG/KG)					
26	295	1.88	0.852	7.88	0.017
27	324	1.78	1.054	8.29	0.025
28	329	1.94	1.065	8.89	0.038

APPENDIX 2

ORGAN WEIGHTS (GRAM)**MALES****END OF RECOVERY**

ANIMAL	BODY W. (GRAM)	BRAIN (GRAM)	HEART (GRAM)	LIVER (GRAM)	THYROIDS (GRAM)
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GROUP 4 (1000 MG/KG)

29	314	1.96	0.977	8.20	0.021
30	315	1.90	0.902	8.86	0.020

MALES**END OF RECOVERY**

ANIMAL	THYMUS (GRAM)	KIDNEYS (GRAM)	ADRENALS (GRAM)	SPLEEN (GRAM)	TESTES (GRAM)
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GROUP 1 (CONTROL)

6	0.393	2.30	0.061	0.484	2.75
7	0.504	2.59	0.074	0.577	3.77
8	0.420	2.21	0.057	0.682	3.09
9	0.359	2.07	0.057	0.524	3.13
10	0.295	2.47	0.054	0.654	3.49

GROUP 4 (1000 MG/KG)

26	0.227	1.89	0.058	0.603	2.88
27	0.409	2.24	0.049	0.584	2.87
28	0.455	2.66	0.083	0.662	3.25
29	0.428	2.14	0.058	0.682	3.60
30	0.458	2.36	0.071	0.525	3.26

MALES**END OF RECOVERY**

ANIMAL	PROSTATE (GRAM)	EPIDIDYMIDES (GRAM)	SEMINAL VES (GRAM)
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GROUP 1 (CONTROL)

6	0.462	0.860	1.052
7	0.480	1.073	1.159
8	0.618	0.995	1.563
9	0.561	0.999	1.454
10	0.450	0.965	1.262

GROUP 4 (1000 MG/KG)

26	0.503	1.033	1.096
27	0.573	0.937	1.219
28	0.663	1.073	1.244
29	0.553	1.077	1.242
30	0.457	0.989	1.136

APPENDIX 2

ORGAN/BODY WEIGHT RATIOS (%)
MALES
END OF TREATMENT

ANIMAL	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYROIDES (%)
GROUP 1 (CONTROL)					
1	269	0.70	0.285	2.63	0.007
2	306	0.66	0.278	2.57	0.009
3	298	0.64	0.290	2.71	0.010
4	319	0.61	0.289	2.62	0.006
5	340	0.60	0.270	2.82	0.007
GROUP 2 (30 MG/KG)					
11	299	0.66	0.300	2.82	0.008
12	282	0.67	0.359	2.69	0.015
13	295	0.65	0.294	2.78	0.008
14	283	0.71	0.309	2.64	0.010
15	295	0.62	0.277	2.89	0.010
GROUP 3 (300 MG/KG)					
16	310	0.63	0.283	3.00	0.010
17	296	0.66	0.301	2.97	0.011
18	293	0.71	0.316	3.00	0.008
19	279	0.69	0.298	3.10	0.007
20	284	0.70	0.324	3.08	0.011
GROUP 4 (1000 MG/KG)					
21	303	0.65	0.310	2.98	0.008
22	302	0.58	0.294	3.10	0.012
23	282	0.71	0.268	2.91	0.010
24	311	0.59	0.302	3.24	0.014
25	282	0.67	0.307	3.10	0.011

MALES
END OF TREATMENT

ANIMAL	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)	SPLEEN (%)	TESTES (%)
GROUP 1 (CONTROL)					
1	0.158	0.72	0.022	0.188	1.05
2	0.133	0.70	0.017	0.225	1.01
3	0.123	0.72	0.018	0.172	0.90
4	0.151	0.72	0.018	0.197	1.09
5	0.152	0.77	0.015	0.194	0.97
GROUP 2 (30 MG/KG)					
11	0.134	0.73	0.019	0.164	1.11
12	0.186	0.73	0.023	0.244	1.01
13	0.120	0.80	0.026	0.190	1.21
14	0.132	0.72	0.020	0.212	1.37
15	0.126	0.76	0.025	0.169	1.08
GROUP 3 (300 MG/KG)					
16	0.133	0.71	0.019	0.217	1.03
17	0.111	0.74	0.019	0.190	1.11
18	0.149	0.81	0.023	0.211	1.11
19	0.118	0.76	0.022	0.226	1.14
20	0.136	0.93	0.021	0.231	1.14

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APPENDIX 2

ORGAN/BODY WEIGHT RATIOS (%)**MALES****END OF TREATMENT**

ANIMAL	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)	SPLEEN (%)	TESTES (%)
GROUP 4 (1000 MG/KG)					
21	0.117	0.71	0.018	0.229	1.08
22	0.184	0.83	0.023	0.222	1.15
23	0.107	0.81	0.021	0.210	1.08
24	0.132	0.84	0.022	0.197	1.09
25	0.094	0.76	0.029	0.205	1.00

MALES**END OF TREATMENT**

ANIMAL	PROSTATE (%)	EPIDIDYMIDES (%)	SEMINAL VES (%)
GROUP 1 (CONTROL)			
1	0.140	0.311	0.342
2	0.141	0.275	0.221
3	0.120	0.280	0.247
4	0.196	0.310	0.340
5	0.124	0.252	0.314
GROUP 2 (30 MG/KG)			
11	0.200	0.280	0.321
12	0.141	0.302	0.255
13	0.200	0.286	0.323
14	0.160	0.348	0.277
15	0.131	0.331	0.280
GROUP 3 (300 MG/KG)			
16	0.122	0.298	0.333
17	0.159	0.294	0.342
18	0.221	0.294	0.263
19	0.206	0.290	0.297
20	0.179	0.332	0.406
GROUP 4 (1000 MG/KG)			
21	0.121	0.317	0.382
22	0.165	0.314	0.284
23	0.156	0.312	0.423
24	0.152	0.313	0.369
25	0.189	0.321	0.420

MALES**END OF RECOVERY**

ANIMAL	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYROIDS (%)
GROUP 1 (CONTROL)					
6	313	0.60	0.283	2.38	0.010
7	396	0.51	0.275	2.43	0.007
8	327	0.58	0.302	2.63	0.007
9	297	0.66	0.314	2.40	0.009
10	329	0.62	0.279	2.71	0.010
GROUP 4 (1000 MG/KG)					
26	295	0.64	0.289	2.67	0.006
27	324	0.55	0.325	2.56	0.008
28	329	0.59	0.324	2.70	0.012

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APPENDIX 2

ORGAN/BODY WEIGHT RATIOS (%)

MALES

END OF RECOVERY

ANIMAL	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYROIDS (%)
GROUP 4 (1000 MG/KG)					
29	314	0.62	0.311	2.61	0.007
30	315	0.60	0.286	2.81	0.006

MALES

END OF RECOVERY

ANIMAL	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)	SPLEEN (%)	TESTES (%)
GROUP 1 (CONTROL)					
6	0.126	0.74	0.019	0.155	0.88
7	0.127	0.65	0.019	0.146	0.95
8	0.128	0.67	0.017	0.209	0.95
9	0.121	0.70	0.019	0.176	1.05
10	0.090	0.75	0.016	0.199	1.06

GROUP 4 (1000 MG/KG)

26	0.077	0.64	0.020	0.204	0.98
27	0.126	0.69	0.015	0.180	0.89
28	0.138	0.81	0.025	0.201	0.99
29	0.136	0.68	0.018	0.217	1.15
30	0.145	0.75	0.023	0.167	1.03

MALES

END OF RECOVERY

ANIMAL	PROSTATE (%)	EPIDIDYIMIDES (%)	SEMINAL VES (%)
GROUP 1 (CONTROL)			
6	0.148	0.275	0.336
7	0.121	0.271	0.293
8	0.189	0.304	0.478
9	0.189	0.336	0.490
10	0.137	0.293	0.384

GROUP 4 (1000 MG/KG)

26	0.171	0.350	0.372
27	0.177	0.289	0.376
28	0.202	0.326	0.378
29	0.176	0.343	0.396
30	0.145	0.314	0.361

APPENDIX 2

NOTOX Project 490627

ORGAN WEIGHTS (GRAM)
FEMALES
END OF TREATMENT

ANIMAL	BODY W. (GRAM)	BRAIN (GRAM)	HEART (GRAM)	LIVER (GRAM)	THYROIDS (GRAM)
GROUP 1 (CONTROL)					
31	184	1.68	0.629	5.57	0.017
32	195	1.77	0.763	5.22	0.025
33	185	1.74	0.675	5.75	0.029
34	185	1.75	0.635	5.49	0.031
35	197	1.79	0.696	5.70	0.026
GROUP 2 (30 MG/KG)					
41	199	1.79	0.692	5.73	0.022
42	177	1.88	0.621	5.39	0.028
43	183	1.80	0.683	5.68	0.027
44	168	1.72	0.565	5.59	0.022
45	187	1.75	0.649	5.98	0.022
GROUP 3 (300 MG/KG)					
46	180	1.85	0.663	5.94	0.020
47	174	1.78	0.526	5.30	0.019
48	172	1.72	0.581	5.64	0.027
49	182	1.75	0.737	6.49	0.032
50	187	1.74	0.660	6.48	0.026
GROUP 4 (1000 MG/KG)					
51	174	1.68	0.592	5.82	0.025
52	178	1.68	0.596	5.71	0.037
53	166	1.88	0.572	5.57	0.035
54	197	1.78	0.638	5.75	0.029
55	160	1.73	0.688	5.92	0.035

FEMALES
END OF TREATMENT

ANIMAL	THYMUS (GRAM)	KIDNEYS (GRAM)	ADRENALS (GRAM)	SPLEEN (GRAM)	OVARIES (GRAM)	UTERUS (GRAM)
GROUP 1 (CONTROL)						
31	0.324	1.49	0.066	0.470	0.115	0.697
32	0.315	1.62	0.073	0.342	0.140	0.333
33	0.441	1.54	0.071	0.424	0.135	0.357
34	0.378	1.43	0.053	0.390	0.095	0.362
35	0.392	1.58	0.084	0.481	0.120	0.488
GROUP 2 (30 MG/KG)						
41	0.296	1.63	0.067	0.456	0.138	0.366
42	0.274	1.42	0.083	0.434	0.118	0.500
43	0.343	1.48	0.075	0.460	0.149	0.545
44	0.262	1.51	0.081	0.471	0.143	0.373
45	0.295	1.41	0.071	0.493	0.141	0.365
GROUP 3 (300 MG/KG)						
46	0.210	1.29	0.074	0.421	0.116	0.358
47	0.302	1.53	0.053	0.331	0.128	0.250
48	0.289	1.50	0.079	0.383	0.147	0.643
49	0.329	1.58	0.068	0.456	0.152	0.713
50	0.302	1.57	0.077	0.536	0.084	0.423

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APPENDIX 2

ORGAN WEIGHTS (GRAM)
FEMALES
END OF TREATMENT

ANIMAL	THYMUS (GRAM)	KIDNEYS (GRAM)	ADRENALS (GRAM)	SPLEEN (GRAM)	OVARIES (GRAM)	UTERUS (GRAM)
GROUP 4 (1000 MG/KG)						
51	0.320	1.42	0.056	0.403	0.119	0.352
52	0.382	1.60	0.066	0.385	0.126	0.520
53	0.319	1.51	0.066	0.387	0.118	0.461
54	0.340	1.56	0.076	0.405	0.125	0.356
55	0.217	1.37	0.091	0.384	0.139	0.556

FEMALES
END OF RECOVERY

ANIMAL	BODY W. (GRAM)	BRAIN (GRAM)	HEART (GRAM)	LIVER (GRAM)	THYROIDES (GRAM)
GROUP 1 (CONTROL)					
36	192	1.77	0.628	5.25	0.025
37	206	1.76	0.799	5.62	0.027
38	207	1.78	0.821	5.79	0.031
39	200	1.80	0.683	4.94	0.032
40	211	1.86	0.731	5.77	0.035
GROUP 4 (1000 MG/KG)					
56	187	1.81	0.588	4.59	0.026
57	189	1.75	0.654	5.14	0.024
58	204	1.86	0.699	5.75	0.024
59	190	1.87	0.675	5.43	0.027
60	203	1.81	0.711	5.64	0.027

FEMALES
END OF RECOVERY

ANIMAL	THYMUS (GRAM)	KIDNEYS (GRAM)	ADRENALS (GRAM)	SPLEEN (GRAM)	OVARIES (GRAM)	UTERUS (GRAM)
GROUP 1 (CONTROL)						
36	0.301	1.51	0.077	0.419	0.130	0.395
37	0.348	1.54	0.090	0.490	0.140	0.403
38	0.364	1.41	0.067	0.514	0.124	0.818
39	0.427	1.49	0.052	0.503	0.118	0.764
40	0.327	1.59	0.092	0.463	0.154	0.350
GROUP 4 (1000 MG/KG)						
56	0.309	1.34	0.059	0.346	0.109	0.377
57	0.277	1.46	0.076	0.477	0.134	0.420
58	0.381	1.42	0.074	0.509	0.166	0.727
59	0.376	1.35	0.058	0.457	0.114	0.444
60	0.445	1.71	0.085	0.514	0.121	0.479

APPENDIX 2

ORGAN/BODY WEIGHT RATIOS (%)
FEMALES
END OF TREATMENT

ANIMAL	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYROIDES (%)
GROUP 1 (CONTROL)					
31	184	0.91	0.342	3.03	0.009
32	195	0.91	0.391	2.68	0.013
33	185	0.94	0.365	3.11	0.016
34	185	0.95	0.343	2.97	0.017
35	197	0.91	0.353	2.89	0.013
GROUP 2 (30 MG/KG)					
41	199	0.90	0.348	2.88	0.011
42	177	1.06	0.351	3.04	0.016
43	183	0.98	0.373	3.10	0.015
44	168	1.02	0.336	3.33	0.013
45	187	0.94	0.347	3.20	0.012
GROUP 3 (300 MG/KG)					
46	180	1.03	0.368	3.30	0.011
47	174	1.02	0.302	3.05	0.011
48	172	1.00	0.338	3.28	0.016
49	182	0.96	0.405	3.57	0.018
50	187	0.93	0.353	3.46	0.014
GROUP 4 (1000 MG/KG)					
51	174	0.97	0.340	3.35	0.014
52	178	0.94	0.335	3.21	0.021
53	166	1.13	0.345	3.35	0.021
54	197	0.90	0.324	2.92	0.015
55	160	1.08	0.430	3.70	0.022

FEMALES
END OF TREATMENT

ANIMAL	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)	SPLEEN (%)	OVARIES (%)	UTERUS (%)
GROUP 1 (CONTROL)						
31	0.176	0.81	0.036	0.255	0.063	0.379
32	0.162	0.83	0.037	0.175	0.072	0.171
33	0.238	0.83	0.038	0.229	0.073	0.193
34	0.204	0.77	0.029	0.211	0.051	0.196
35	0.199	0.80	0.043	0.244	0.061	0.248
GROUP 2 (30 MG/KG)						
41	0.149	0.82	0.034	0.229	0.069	0.184
42	0.155	0.80	0.047	0.245	0.067	0.282
43	0.187	0.81	0.041	0.251	0.081	0.298
44	0.156	0.90	0.048	0.280	0.085	0.222
45	0.158	0.75	0.038	0.264	0.075	0.195
GROUP 3 (300 MG/KG)						
46	0.117	0.72	0.041	0.234	0.064	0.199
47	0.174	0.88	0.030	0.190	0.074	0.144
48	0.168	0.87	0.046	0.223	0.085	0.374
49	0.181	0.87	0.037	0.251	0.084	0.392
50	0.161	0.84	0.041	0.287	0.045	0.226

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APPENDIX 2

ORGAN/BODY WEIGHT RATIOS (%)

FEMALES

END OF TREATMENT

ANIMAL	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)	SPLEEN (%)	OVARIES (%)	UTERUS (%)
GROUP 4 (1000 MG/KG)						
51	0.184	0.81	0.032	0.232	0.068	0.202
52	0.215	0.90	0.037	0.216	0.071	0.292
53	0.192	0.91	0.040	0.233	0.071	0.278
54	0.173	0.79	0.039	0.206	0.063	0.181
55	0.136	0.85	0.057	0.240	0.087	0.348

FEMALES

END OF RECOVERY

ANIMAL	BODY W. (GRAM)	BRAIN (%)	HEART (%)	LIVER (%)	THYROIDS (%)
GROUP 1 (CONTROL)					
36	192	0.92	0.327	2.73	0.013
37	206	0.85	0.388	2.73	0.013
38	207	0.86	0.397	2.80	0.015
39	200	0.90	0.342	2.47	0.016
40	211	0.88	0.346	2.74	0.017
GROUP 4 (1000 MG/KG)					
56	187	0.97	0.314	2.46	0.014
57	189	0.93	0.346	2.72	0.013
58	204	0.91	0.343	2.82	0.012
59	190	0.99	0.355	2.86	0.014
60	203	0.89	0.350	2.78	0.013

FEMALES

END OF RECOVERY

ANIMAL	THYMUS (%)	KIDNEYS (%)	ADRENALS (%)	SPLEEN (%)	OVARIES (%)	UTERUS (%)
GROUP 1 (CONTROL)						
36	0.157	0.78	0.040	0.218	0.068	0.206
37	0.169	0.75	0.044	0.238	0.068	0.196
38	0.176	0.68	0.032	0.248	0.060	0.395
39	0.214	0.74	0.026	0.252	0.059	0.382
40	0.155	0.76	0.044	0.219	0.073	0.166
GROUP 4 (1000 MG/KG)						
56	0.165	0.72	0.032	0.185	0.058	0.202
57	0.147	0.77	0.040	0.252	0.071	0.222
58	0.187	0.69	0.036	0.250	0.081	0.356
59	0.198	0.71	0.031	0.241	0.060	0.234
60	0.219	0.84	0.042	0.253	0.060	0.236

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KEY TO MISSING VALUES/REMARKS CLINICAL LABORATORY INVESTIGATIONS

End of Treatment

Haematology:

Animal(s):

8	---	= Insufficient sample for APTT
3, 15, 25, 46		Differential leucocyte count was also performed manually because of a technical error / an abnormal plot in the automated count and these results are reported

Clinical Biochemistry: No remarks

End of Recovery

Haematology:

Animal(s):

6, 10, 26, 56	---	= Citrate sample clotted
6, 9	---	= EDTA sample clotted

Clinical Biochemistry: No remarks

APPENDIX 3

SUMMARY OF DOSE RANGE FINDING STUDY

Summary of Dose Range Finding Study

Study Plan Treatment: 05 March 2009 to 09 March 2009
Necropsy: 09 March 2009

Aim of the study

In order to set the dose levels for the main study (NOTOX Project 490627), a pilot study was performed NOTOX Project 490628).

Guidelines

No guidelines were applicable as this study was intended for dose level selection purposes only.

Materials and methods

If not mentioned otherwise, test system, procedures and techniques were identical to those used during the main study.

Test System/Animal husbandry

Number of rats/group 3 females (allocated at random and identified by ear- and tailmark),
Age at start of treatment approximately 6 weeks
Room number 7
Housing Conditions Group housing with 3 animals per sex.
Actual temperature range: 19.6 - 20.8°C.
Actual relative humidity range: 30 - 59%.

Treatment

Duration of treatment 5 days
Dose levels 500 and 1000 mg/kg body weight/day.
No chemical analyses of dose preparations were conducted.
Dose volume 5 mL/kg body weight/day
Vehicle Polyethylene glycol 400.

Observations

Clinical signs: At least once daily.
Mortality: At least twice daily.
Body weights: On days 1 and 5.
Food consumption: Over days 1-5.

Pathology

Necropsy: On day 5 (scheduled necropsy): all animals. No organs were fixed.
Organ weights: Terminal body weight, kidney, and liver weight.

List of protocol deviations

There were no deviations from the protocol.

Results

No signs of toxicity were noted at any dose level.

Conclusion

Based on the results of this range finding study, dose levels for the main study were: 30, 300 and 1000 mg/kg body weight/day.

APPENDIX 4

ANALYTICAL REPORT

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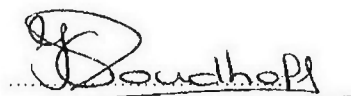
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2. REPORT APPROVAL

PRINCIPAL SCIENTIST:

Dr. K.A. Oudhoff
(Analytical Chemistry)


.....Oudhoff

Date: 26 May 2009

3. SUMMARY

The purpose of this part of the study was to determine the accuracy of preparation, homogeneity and stability of the test substance in formulations.

The concentrations analysed in the formulations of Group 2, Group 3 and Group 4 were in agreement with target concentrations (i.e. mean accuracies between 85% and 115%).

No test substance was detected in the Group 1 formulations.

The formulations of Group 2 and Group 4 were homogeneous (i.e. coefficient of variation $\leq 10\%$).

Formulations at the entire range were stable when stored at room temperature for at least 6 hours.

4. INTRODUCTION

4.1. Preface

Study plan
(analytical study)

Start : 23 March 2009
Completion : 06 May 2009

4.2. Aim of the study

The purpose of the analytical study was to determine the accuracy of preparation, homogeneity and stability of the test substance in formulations.

5. MATERIALS AND METHODS

5.1. Reagents

Water

Tap water purified by a Milli-Q water purification system
(Millipore, Bedford, MA, USA)

Acetonitrile

Biosolve, Valkenswaard, The Netherlands.

Tetrahydrofuran (THF)

VWR International, Leuven, Belgium

All reagents were of analytical grade, unless specified otherwise.

5.2. Samples

Accuracy, homogeneity and stability were determined for formulations prepared after the in-life phase. The formulations were prepared similarly as those used during the in-life phase.

Duplicate samples (approximately 500 mg), which were taken from the formulations using a pipette, were accurately weighed into volumetric flasks of 20, 50 or 100 ml. For determination of accuracy, samples were taken at 50% height or at 90%, 50% and 10% height. The latter set of samples was also used for the determination of the homogeneity of the formulations. For determination of stability, additional samples were taken at 50% height and stored at room temperature for 6 hours.

The volumetric flasks were filled up to the mark with THF. In order to dissolve the test substance the solutions were ultrasonicated for 15 minutes. If necessary, the solutions were diluted with THF to obtain concentrations close to the calibrated range. The solutions were then diluted with water to obtain an end solution of 50/50 (v/v) THF/water and analysed.

5.3. Analytical method

5.3.1. Analytical conditions

Quantitative analysis was based on the analytical method validated for the test substance in NOTOX project 490629.

Analytical conditions:

Instrument

Alliance Separation Module 2695
(Waters, Milford, MA, USA)

Detector

Dual λ Absorbance Detector 2487 (Waters)

Column

Symmetry Shield RP-18, 100 mm \times 4.6 mm i.d.,
dp = 3.5 μ m (Waters)

Column temperature

35°C \pm 1°C

Injection volume

10 μ l

Mobile phase

95/5 (v/v) acetonitrile/water

Flow

1 ml/min

UV detection

235 nm

5.3.2. Preparation of the calibration solutions

Stock and spiking solutions

Stock and spiking solutions of the test substance were prepared in THF at a concentration of 1330 or 1900 mg/l. In order to dissolve the test substance the solutions were ultrasonicated for 10 minutes.

Calibration solutions

Calibration solutions in the concentration range 5 – 75 mg/l were prepared from two stock solutions. The end solution of the calibration solutions was 50/50 (v/v) THF/water.

Procedural recovery samples

Approximately 500 mg blank vehicle was spiked with the test substance at a target concentration of 1 or 190 mg/g. The accuracy samples were treated similarly as the test samples (see paragraph 5.2 'Samples').

5.3.3. Sample injections

Calibration solutions were injected in duplicate. Test samples and procedural recovery samples were analysed by single injection.

5.4. Electronic data capture

System control, data acquisition and data processing were performed using the following programme:

- Empower version 5.00 (Waters, Milford, MA, USA).

5.5. Formulas

Response (R)

Peak area test substance [units]

Calibration curve

$$R = a C_N + b$$

where:

C_N = nominal concentration [mg/l]

a = slope [units \times l/mg]

b = intercept [units]

Analysed concentration (C_A)

$$C_A = \frac{(R - b)}{a} \times \frac{V \times d}{w} \quad [\text{mg/g}]$$

where:

w = weight sample [mg]

V = volume volumetric flask [ml]

d = dilution factor

Recovery

$$\frac{C_A}{C_N} \times 100\%$$

where:

C_N = nominal concentration [mg/g]

Accuracy

$$\frac{C_A}{C_T} \times 100\%$$

where:

 C_T = target concentration [mg/g]

Relative difference (relative diff.)

$$\frac{C_t - C_0}{C_0} \times 100\%$$

where:

 C_t = mean concentration of stored samples [mg/g] C_0 = mean concentration of non-stored samples [mg/g]

5.6. Specifications

Preparation of formulations was considered acceptable if the mean accuracy was in the range 85% - 115% of the target concentration and if the coefficient of variation was $\leq 10\%$. Formulations were considered stable if the relative difference between the stored and freshly taken samples was $\leq 10\%$.

6. RESULTS

6.1. Calibration curves

A calibration curve was constructed using five concentrations. For each concentration, two responses were used. Linear regression analysis was performed using the least squares method with a $1/\text{concentration}^2$ weighting factor. The coefficient of correlation (r) was > 0.99 .

6.2. Samples

6.2.1. Procedural recovery samples

The results for the procedural recovery samples are given in Table 1.

Mean recoveries of the procedural recovery samples were 108% and 109%. Because the criterion that mean recoveries should be between 85% and 115% was met, the results for the test samples were accepted.

6.2.2. Test samples

The results of the test samples are given in Table 2 and 3.

In the Group 1 formulations, no test substance was detected.

The concentrations analysed in the formulations of Group 2, Group 3 and Group 4 were in agreement with target concentrations (i.e. mean accuracies between 85% and 115%).

The formulations of Group 2 and Group 4 were homogeneous (i.e. coefficient of variation $\leq 10\%$).

Analysis of Group 2 and Group 4 formulations after storage yielded a relative difference of $\leq 10\%$. Based on this, the formulations were found to be stable during storage at room temperature for at least 6 hours.

TABLES

Table 1 Procedural recovery samples

Date of preparation [dd-mm-yy]	Date of analysis [dd-mm-yy]	Target concentration [mg/g]	Nominal concentration [mg/g]	Analysed concentration [mg/g]	Recovery [%]	Mean recovery [%]
05-05-09	05-05-09	1	1.00	1.08	108	108
			0.989	1.07	108	
05-05-09	05-05-09	190	191	208	109	109
			186	202	109	

Table 2 Accuracy and homogeneity test

Group	Date of analysis [dd-mm-yy]	Sample position	Concentration [mg/g]		Accuracy [%]		Homogeneity (coefficient of variation) [%]
			Target	Analysed	Individual	Mean	
1	05-05-09	50% height	0.00 0.00	n.d. n.d.	n.a. n.a.	n.a.	n.a.
2	05-05-09	90% height	5.34 5.34	6.09 6.08	114 114	112	2.7
		50% height	5.34 5.34	6.09 5.78	114 108		
		10% height	5.34 5.34	6.11 5.79	114 108		
3	05-05-09	50% height	53.7 53.7	58.0 59.5	108 111	109	n.a.
4	05-05-09	90% height	182 182	201 203	110 112	110	1.8
		50% height	182 182	194 200	107 110		
		10% height	182 182	196 203	108 112		

n.d. Not detected.

n.a. Not applicable.

Table 3 Stability test

Group	Date of analysis [dd-mm-yy]	Analysed concentration [mg/g]		Relative diff. [%]
		t=0 ¹	t=6 hours	
2	05-05-09	5.99	5.63 ²	-6.1
4	05-05-09	200	199 ³	-0.28

¹ Mean of six samples at t=0 taken at 10%, 50% and 90% height (see Table 2).² Mean of two samples at t=6 hours taken at 50% height. Individual results were 5.56 and 5.69 mg/g.³ Mean of two samples at t=6 hours taken at 50% height. Individual results were 197 and 201 mg/g.

APPENDIX 5

HISTOPATHOLOGY REPORT

PATHOLOGY REPORT

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TOX : 490627

TEST ITEM : ☐ ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

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TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData©System	V6.2d1

AUTHENTICATION

I, the undersigned hereby declare that the histopathology data in this report were compiled by me, and that they reflect accurately the primary data records.

Hetty van den Brink D.V.M.
Toxicologic Pathologist



26 May 2009

NOTOX B.V.
P.O. Box 3476
5203 DL 's-Hertogenbosch
The Netherlands

PATHOLOGY REPORT
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TEST ITEM	: []	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

SUMMARY

Pathomorphologic examination was performed on 60 Wistar (Han) rats (30 males, 30 females) which had been subjected to a 28-day oral (gavage) toxicity study with the test item SH-1.

The rats were assigned to four dose groups, Groups 1 and 4 containing ten males and ten females, Groups 2 and 3 containing five males and five females. The test item was administered once daily by gavage at doses of 30, 300 and 1000 mg/kg (dose Groups 2, 3 and 4 respectively) for 28 days. The rats of the control Group 1 received the vehicle, polyethylene glycol 400, alone. At the end of the treatment period five animals of each sex from all groups were killed and subjected to complete necropsies. These animals are identified in this report as the main groups. The remaining five males and five females of Groups 1 and 4 were necropsied following a 14-day treatment-free period. These animals are identified in this report as the recovery groups.

All rats were necropsied. Histopathologic examination was performed on an extensive list of organs and tissues from Group 1 and 4 rats, as well as all organs with macroscopic findings from all rats.

There were no unscheduled deaths.

There were no treatment-related macroscopic or microscopic findings.

There was no morphologic evidence of toxicity to the test item [].

PATHOLOGY REPORT
PRINCIPAL SECTION

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TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

METHODS

Study Design

Group	Dose level mg/kg/day	Number of animals		Animal numbers	
		Males	Females	Males	Females
1 Main group	0 (vehicle)	5	5	1-5	31-35
1 Recovery group	0 (vehicle)	5	5	6-10	36-40
2 Main group	30	5	5	11-15	41-45
3 Main group	300	5	5	16-20	46-50
4 Main group	1000	5	5	21-25	51-55
4 Recovery group	1000	5	5	26-30	56-60

Administration of the Test Item

Groups of 5 male and 5 female Wistar (Han) rats received 0, 30, 300, or 1000 mg/kg body weight/day of ☐ by oral gavage for 28 days. Rats of the control Group 1 received the vehicle, polyethylene glycol 400, specific gravity 1.125, alone. These animals are identified in this report as the main groups.

The recovery groups of 5 male and 5 female Wistar rats received the vehicle or 1000 mg/kg body weight/day of ☐ by oral gavage for 28 days, followed by a 14-day treatment-free recovery period.

Necropsy and Histopathology

At the end of the assigned study periods, the rats were killed by exsanguination following anesthesia by iso-flurane. Complete necropsies were performed on all rats.

The terminal body weight and the weights of the adrenal glands, brain, epididymides, heart, kidneys, liver, testes, thymus and uterus were recorded at the scheduled necropsies. Prostate, seminal vesicles and thyroid including parathyroid were weighed after fixation for at least 24 hours.

Representative tissue samples of the following organs were preserved in 4% phosphate buffered neutral formaldehyde solution (10% formalin), testes, epididymides, eyes including optic nerve and Harderian gland were initially fixed in Modified Davidson's solution.

Adrenal glands (2), [aorta], bone - sternum (1) and femur including joint (1), bone marrow - sternal (1), brain (cerebellum, mid-brain, cortex), [clitoral glands], epididymides (2), [oesophagus], eyes (2) with [optic nerve and Harderian glands], heart (1), [identification marks], kidneys (2), [lacrimial glands - exorbital], large intestine (3) - cecum, colon and rectum; [larynx], liver (2), lungs (2), lymph nodes - mandibular (2) and mesenteric (1), [female mammary gland area], [nasopharynx], ovaries (2), [pancreas], pituitary gland (1), [preputial glands], prostate gland (1), [salivary glands - mandibular and sublingual], sciatic nerve (1), seminal vesicles (2) including coagulation gland (2), skeletal muscle (1), [skin], small intestine (3) - duodenum, jejunum and ileum with Peyer's patches; spinal cord (3) - cervical, midthoracic and lumbar; spleen (1), stomach (1), testes (2), thymus (1), thyroid glands (2) with parathyroid glands (2), [tongue], trachea (1), urinary bladder (1), uterus (3), uterine cervix (1), vagina (1) and all organs or tissues with macroscopic abnormalities.

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SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

METHODS

Following fixation, organs (except those listed in brackets) from group 1 and 4 rats of the main study as well as all organs with macroscopic abnormalities from all rats, were trimmed, processed and embedded in paraffin wax. Sections were cut at a thickness of 2-4 micrometers and stained with hematoxylin and eosin. Numbers of sections made are given in parentheses.

The sections were examined by light microscopy in the period 24 April 2009 - 6 May 2009.

Data compilation

The animal data and macroscopic findings were electronically transferred from the necropsy raw data files of Toxdata system® into the computer system PathData® where the microscopic findings were recorded by the undersigned pathologist using on-line input under pathology number: 20907 BRH.

All macroscopic and microscopic findings are given for each animal in text form under "Text of Gross and Microscopic Findings". The incidence of microscopic findings is also presented in tabular form: "Incidence table - all microscopic findings". Incidence tables are created by computer.

Histopathological changes were described according to distribution, severity and morphological character.

Severity scores were assigned as follows:

GRADE 1 = Minimal / very few / very small

GRADE 2 = Slight / few / small

GRADE 3 = Moderate / moderate number / moderate size

GRADE 4 = Marked / many / large

GRADE 5 = Massive / extensive number / extensive size

P = Finding present, severity not scored

(= Finding unilateral in paired organs

In the case of findings in bilaterally affected paired organs, the severity score of the worst affected organ was recorded and a comment was made listing the severity score in the contralateral organ.

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PRINCIPAL SECTION

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TEST ITEM	: []	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

RESULTS

Mortality

All rats survived the scheduled duration of the study.

Macroscopic Findings

All macroscopic findings recorded were considered to be spontaneous in nature.

Microscopic Findings

All microscopic findings recorded were considered to be within the normal range of background pathology encountered in Wistar rats of this age and strain.

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TEST ITEM	: []	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

CONCLUSIONS

Treatment-related morphologic alterations following the administration of [] by once daily gavage to Wistar rats at doses up to 1000 mg/kg/day for 28 days, followed by a 14-day recovery period, were not in evidence.

PATHOLOGY REPORT

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TEST ITEM : 
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

EXPLANATION OF CODES AND SYMBOLS

CODES AND SYMBOLS USED AT ANIMAL LEVEL:

M = Male animal
F = Female animal
K0 = Terminal sacrifice group
R1...R9 = Recovery / post-treatment group 1...9

CODES AND SYMBOLS USED AT ORGAN LEVEL:

G = Gross observation checked off histologically
' = Histologic examination not required
+ = Organ examined, findings present
- = Organ examined, no pathologic findings noted (AOFT* only)
(* Animal Organ Finding Table)
(= Only one of paired organs examined/present

CODES AND SYMBOLS USED AT FINDING LEVEL:

GRADE 1 = Minimal / very few / very small
GRADE 2 = Slight / few / small
P = Finding present, severity not scored
(= Finding unilateral in paired organs

PATHOLOGY REPORT
SUMMARY TABLES

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TOX : 490627

TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: K0

Incidence table - All microscopic findings

	SEX :					MALE
DOSE GROUP:		01	02	03	04	
NO. ANIMALS:		5	5	5	5	
BRAIN	:	5	-	-	5	
SPINAL CORD - CERV.	:	5	-	-	5	
SPINAL CORD - THOR.	:	5	-	-	5	
SPINAL CORD - LUMBAR	:	5	-	-	5	
SCIATIC NERVE	:	5	-	-	5	
- Myelin fragmentation:		2	-	-	-	
HEART	:	5	-	-	5	
- Inflamm. lymphocytic:		1	-	-	-	
TRACHEA	:	5	-	-	5	
LUNG	:	5	-	-	5	
- Mineraliz., vascular:		2	-	-	-	
- Alveolar inflamm.	:	-	-	-	1	
- Lymphoid hyperplasia:		2	-	-	-	
- Osseous metaplasia	:	1	-	-	-	
STOMACH	:	5	-	-	5	
DUODENUM	:	5	-	-	5	
JEJUNUM	:	5	-	-	5	
ILEUM	:	5	-	-	5	
PEYER'S PATCHES	:	5	-	-	5	
- Mineraliz., focal	:	-	-	-	1	
CECUM	:	5	-	-	5	
COLON	:	5	-	-	5	

PATHOLOGY REPORT
SUMMARY TABLES

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TEST ITEM : ☐ ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: K0

Incidence table - All microscopic findings

SEX :						MALE
DOSE GROUP:		01	02	03	04	
NO. ANIMALS:		5	5	5	5	
RECTUM	:	5	-	-	5	
LIVER	:	5	-	-	5	
- Hemopoietic cells	:	-	-	-	1	
- Inflamm. cell foci	:	5	-	-	5	
- Inflamm. peribiliary:		-	-	-	1	
KIDNEYS	:	5	-	-	5	
- Cyst(s)	:	1	-	-	-	
- Dilatation, tubular	:	-	-	-	1	
- Hyaline droplets	:	2	-	-	-	
- Inflamm., interstit.:		2	-	-	-	
- Basophilia, tubular	:	3	-	-	2	
URINARY BLADDER	:	5	-	-	5	
TESTES	:	5	-	-	5	
EPIDIDYMIDES	:	5	-	-	5	
PROSTATE GLAND	:	5	-	-	5	
- Inflamm. lymphocytic:		2	-	-	-	
COAGULATING GLANDS	:	5	-	-	4	
SEMINAL VESICLES	:	5	-	-	5	
- Reduced contents	:	1	-	-	-	
PITUITARY GLAND	:	5	-	-	5	
- Cyst	:	-	-	-	1	
THYROID GLAND	:	5	-	-	5	
PARATHYROID GLANDS	:	5	-	-	5	
ADRENAL GLANDS	:	5	-	-	5	
- Vacuolation, z.fas.	:	-	-	-	1	

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TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: K0

Incidence table - All microscopic findings

	SEX :					MALE
	DOSE GROUP:	01	02	03	04	
	NO. ANIMALS:	5	5	5	5	
SPLEEN	:	5	—	—	5	
— Hemopoietic foci	:	4	—	—	4	
BONE MARROW — STERN.	:	5	—	—	5	
BONE MARROW, FEMUR	:	5	—	—	5	
THYMUS	:	5	—	—	5	
— Lymphoid atrophy	:	—	—	—	1	
MESENT. LYMPH NODE	:	5	—	—	5	
MANDIB. LYMPH NODES	:	5	—	—	5	
— Macrophage foci	:	1	—	—	—	
— Plasmacytosis	:	—	—	—	1	
SKELETAL MUSCLE	:	5	—	—	5	
BONE — STERNUM	:	5	—	—	5	
JOINTS, KNEE	:	5	—	—	5	
EYES	:	5	—	—	5	
FEMUR	:	5	—	—	5	

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TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.
PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2dl

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: K0

Incidence table - All microscopic findings

SEX :						FEMALE
DOSE GROUP:		01	02	03	04	
NO. ANIMALS:		5	5	5	5	
BRAIN	:	5	—	—	5	
SPINAL CORD — CERV.	:	5	—	—	5	
SPINAL CORD — THOR.	:	5	—	—	5	
SPINAL CORD — LUMBAR	:	5	—	—	5	
SCIATIC NERVE	:	5	—	—	5	
— Myelin fragmentation:	:	1	—	—	1	
HEART	:	5	—	—	5	
— Inflamm. lymphocytic:	:	—	—	—	2	
TRACHEA	:	5	—	—	5	
LUNG	:	5	—	—	5	
— Mineraliz., vascular:	:	1	—	—	—	
— Alveolar inflamm.	:	1	—	—	—	
— Lymphoid hyperplasia:	:	—	—	—	4	
STOMACH	:	5	—	—	5	
DUODENUM	:	5	—	—	5	
JEJUNUM	:	5	—	—	5	
ILEUM	:	5	—	—	5	
PEYER'S PATCHES	:	5	—	—	5	
CECUM	:	5	—	—	5	
COLON	:	5	—	—	5	
RECTUM	:	5	—	—	5	

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TEST ITEM : ☐
 TEST SYSTEM : RAT, 28 day with rec., Gavage
 SPONSOR : Cheil Industries Inc.

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 DATE : 26-MAY-09
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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: K0

Incidence table - All microscopic findings

SEX :						FEMALE
DOSE GROUP:		01	02	03	04	
NO. ANIMALS:		5	5	5	5	
LIVER :		5	-	-	5	
- Hemopoietic cells :		-	-	-	1	
- Inflamm. cell foci :		5	-	-	5	
KIDNEYS :		5	-	-	5	
- Hyaline cast(s) :		-	-	-	1	
- Basophilia, tubular :		1	-	-	1	
URINARY BLADDER :		5	-	-	5	
OVARIES :		5	-	-	5	
UTERUS :		5	-	1	5	
- Estrus epithelium :		-	-	1	1	
- Proestrus epithelium:		1	-	-	-	
CERVIX :		5	-	-	5	
VAGINA :		5	-	-	5	
PITUITARY GLAND :		5	-	-	5	
THYROID GLAND :		5	-	-	5	
PARATHYROID GLANDS :		5	-	-	5	
ADRENAL GLANDS :		5	-	-	5	
SPLEEN :		5	-	-	5	
- Hemopoietic foci :		4	-	-	2	
- Hemosiderin pigment :		4	-	-	5	
BONE MARROW - STERN. :		5	-	-	5	
BONE MARROW, FEMUR :		5	-	-	5	
THYMUS :		5	-	-	5	

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NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: K0

Incidence table - All microscopic findings

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO. ANIMALS:	5	5	5	5	
MESENT. LYMPH NODE :	5	-	-	5	
MANDIB. LYMPH NODES :	5	-	-	5	
SKELETAL MUSCLE :	5	-	-	5	
BONE - STERNUM :	5	-	-	5	
JOINTS, KNEE :	5	-	-	5	
EYES :	5	-	-	5	
FEMUR :	5	-	-	5	

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TEST SYSTEM : RAT, 28 day with rec., Gavage DATE : 26-MAY-09
SPONSOR : Cheil Industries Inc. PathData©System V6.2d1

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX

STATUS AT NECROPSY: R1

Incidence table - All microscopic findings

SEX :					FEMALE
DOSE GROUP:	01	02	03	04	
NO. ANIMALS:	5	-	-	5	
UTERUS :	1	-	-	2	
- Estrus epithelium :	-	-	-	2	
- Proestrus epithelium:	1	-	-	-	
CLITORAL GLANDS :	2	-	-	-	
- Dilated gland :	2	-	-	-	

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TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
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PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, CONTROL

ANIMAL NUMBER :

	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MR1	MR1	MR1	MR1	MR1
BRAIN	-	-	-	-	-	+	+	+	+	+
SPINAL CORD - CERV.	-	-	-	-	-	+	+	+	+	+
SPINAL CORD - THOR.	-	-	-	-	-	+	+	+	+	+
SPINAL CORD - LUMBAR	-	-	-	-	-	+	+	+	+	+
SCIATIC NERVE	-	+	-	+	-	+	+	+	+	+
- Myelin fragmentation	.	1.	.	1.
HEART	-	+	-	-	-	+	+	+	+	+
- Inflamm. lymphocytic	.	1.
TRACHEA	-	-	-	-	-	+	+	+	+	+
LUNG	+	+	+	+	-	+	+	+	+	+
- Mineraliz., vascular	1.	.	1.
- Lymphoid hyperplasia	.	1.	.	2.
- Osseous metaplasia	1.
STOMACH	-	-	-	-	-	+	+	+	+	+
DUODENUM	-	-	-	-	-	+	+	+	+	+
JEJUNUM	-	-	-	-	-	+	+	+	+	+
ILEUM	-	-	-	-	-	+	+	+	+	+
PEYER'S PATCHES	-	-	-	-	-	+	+	+	+	+
CECUM	-	-	-	-	-	+	+	+	+	+
COLON	-	-	-	-	-	+	+	+	+	+
RECTUM	-	-	-	-	-	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+
- Inflamm. cell foci	1.	1.	1.	1.	1.

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TEST ITEM : 
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, CONTROL

ANIMAL NUMBER :

	1	2	3	4	5	6	7	8	9	10
	MKO	MKO	MKO	MKO	MKO	MR1	MR1	MR1	MR1	MR1
KIDNEYS	+	+	-	+	+	'	'	'	'	'
- Cyst(s)	.	(2.	.	.	.					
- Hyaline droplets	1.	.	.	.	1.					
- Inflamm., interstit.	.	(1.	.	(1.	.					
- Basophilia, tubular	1.	(1.	.	.	(1.					
.....										
URINARY BLADDER	-	-	-	-	-	'	'	'	'	'
.....										
TESTES	-	-	-	-	-	'	'	'	'	'
.....										
EPIDIDYIMIDES	-	-	-	-	-	'	'	'	'	'
.....										
PROSTATE GLAND	-	+	+	-	-	'	'	'	'	'
- Inflamm. lymphocytic	.	1.	1.	.	.					
.....										
COAGULATING GLANDS	-	-	-	-	-	'	'	'	'	'
.....										
SEMINAL VESICLES	-	+G	-	-	-	'	'	'	'	'
- Reduced contents	.	(1.	.	.	.					
.....										
PITUITARY GLAND	-	-	-	-	-	'	'	'	'	'
.....										
THYROID GLAND	-	-	-	-	-	'	'	'	'	'
.....										
PARATHYROID GLANDS	-	-	-	-	-	'	'	'	'	'
.....										
ADRENAL GLANDS	-	-	-	-	-	'	'	'	'	'
.....										
SPLEEN	+	-	+	+	+	'	'	'	'	'
- Hemopoietic foci	1.	.	1.	1.	2.					
.....										
BONE MARROW - STERN.	-	-	-	-	-	'	'	'	'	'
.....										
BONE MARROW, FEMUR	-	-	-	-	-	'	'	'	'	'
.....										
THYMUS	-	-	-	-	-	'	'	'	'	'
.....										
MESENT. LYMPH NODE	-	-	-	-	-	'	'	'	'	'
.....										

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TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, CONTROL

ANIMAL NUMBER :

	1	2	3	4	5	6	7	8	9	10
	MK0	MK0	MK0	MK0	MK0	MR1	MR1	MR1	MR1	MR1
MANDIB. LYMPH NODES	+	-	-	-	-	'	'	'	'	'
- Macrophage foci	(1.					
SKELETAL MUSCLE	-	-	-	-	-	'	'	'	'	'
BONE - STERNUM	-	-	-	-	-	'	'	'	'	'
JOINTS, KNEE	-	-	-	-	-	'	'	'	'	'
EYES	-	-	-	-	-	'	'	'	'	'
FEMUR	-	-	-	-	-	'	'	'	'	'

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INDIVIDUAL ANIMAL DATA

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TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, CONTROL

ANIMAL NUMBER :

	31	32	33	34	35	36	37	38	39	40
	FK0	FK0	FK0	FK0	FK0	FR1	FR1	FR1	FR1	FR1
BRAIN	-	-	-	-	-	+	+	+	+	+
SPINAL CORD - CERV.	-	-	-	-	-	+	+	+	+	+
SPINAL CORD - THOR.	-	-	-	-	-	+	+	+	+	+
SPINAL CORD - LUMBAR	-	-	-	-	-	+	+	+	+	+
SCIATIC NERVE	-	-	-	-	+	+	+	+	+	+
- Myelin fragmentation	1.					
HEART	-	-	-	-	-	+	+	+	+	+
TRACHEA	-	-	-	-	-	+	+	+	+	+
LUNG	-	-	+	+	-	+	+	+	+	+
- Mineraliz., vascular	.	.	.	1.	.					
- Alveolar inflamm.	.	.	1.	.	.					
STOMACH	-	-	-	-	-	+	+	+	+	+
DUODENUM	-	-	-	-	-	+	+	+	+	+
JEJUNUM	-	-	-	-	-	+	+	+	+	+
ILEUM	-	-	-	-	-	+	+	+	+	+
PEYER'S PATCHES	-	-	-	-	-	+	+	+	+	+
CECUM	-	-	-	-	-	+	+	+	+	+
COLON	-	-	-	-	-	+	+	+	+	+
RECTUM	-	-	-	-	-	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+
- Inflamm. cell foci	1.	1.	1.	1.	1.					

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TEST ITEM : ☐ ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

DOSE GROUP : 01, CONTROL

ANIMAL NUMBER :

	31	32	33	34	35	36	37	38	39	40
	FK0	FK0	FK0	FK0	FK0	FR1	FR1	FR1	FR1	FR1
KIDNEYS	-	+	-	-	-	'	'	'	'	'
- Basophilia, tubular	.	(1.	.	.	.					
.....										
URINARY BLADDER	-	-	-	-	-	'	'	'	'	'
.....										
OVARIES	-	-	-	-	-	'	'	'	'	'
.....										
UTERUS	+G	-	-	-	-	'	'	+G	'	'
- Proestrus epithelium	P.			P.		
.....										
CERVIX	-	-	-	-	-	'	'	'	'	'
.....										
VAGINA	-	-	-	-	-	'	'	'	'	'
.....										
CLITORAL GLANDS	'	'	'	'	'	+G	'	'	+G	'
- Dilated gland						(2.		(1.		
.....										
PITUITARY GLAND	-	-	-	-	-	'	'	'	'	'
.....										
THYROID GLAND	-	-	-	-	-	'	'	'	'	'
.....										
PARATHYROID GLANDS	-	(-	-	-	-	'	'	'	'	'
.....										
ADRENAL GLANDS	-	-	-	-	-	'	'	'	'	'
.....										
SPLEEN	+	+	+	+	+	'	'	'	'	'
- Hemopoietic foci	1.	.	1.	1.	1.					
- Hemosiderin pigment	1.	1.	.	1.	1.					
.....										
BONE MARROW - STERN.	-	-	-	-	-	'	'	'	'	'
.....										
BONE MARROW, FEMUR	-	-	-	-	-	'	'	'	'	'
.....										
THYMUS	-	-	-	-	-	'	'	'	'	'
.....										
MESENT. LYMPH NODE	-	-	-	-	-	'	'	'	'	'
.....										
MANDIB. LYMPH NODES	-	-	-	-	-	'	'	'	'	'
.....										

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PATHOL. NO.: 20907 BRH
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 01, CONTROL

ANIMAL NUMBER :

	31	32	33	34	35	36	37	38	39	40
	FK0	FK0	FK0	FK0	FK0	FR1	FR1	FR1	FR1	FR1
SKELETAL MUSCLE	-	-	-	-	-					
BONE - STERNUM	-	-	-	-	-					
JOINTS, KNEE	-	-	-	-	-					
EYES	-	-	-	-	-					
FEMUR	-	-	-	-	-					

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

DOSE GROUP : 03, 300 MG/KG

ANIMAL NUMBER :

	16	17	18	19	20	46	47	48	49	50
	MKO	MKO	MKO	MKO	MKO	FKO	FKO	FKO	FKO	FKO

UTERUS

- Estrus epithelium

									+G	
									P.	

.....

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TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER :

	21	22	23	24	25	26	27	28	29	30
	MK0	MK0	MK0	MK0	MK0	MR1	MR1	MR1	MR1	MR1
BRAIN	-	-	-	-	-	-	-	-	-	-
SPINAL CORD - CERV.	-	-	-	-	-	-	-	-	-	-
SPINAL CORD - THOR.	-	-	-	-	-	-	-	-	-	-
SPINAL CORD - LUMBAR	-	-	-	-	-	-	-	-	-	-
SCIATIC NERVE	-	-	-	-	-	-	-	-	-	-
HEART	-	-	-	-	-	-	-	-	-	-
TRACHEA	-	-	-	-	-	-	-	-	-	-
LUNG	-	-	+	-	-	-	-	-	-	-
- Alveolar inflamm.	.	.	1.
STOMACH	-	-	-	-	-	-	-	-	-	-
DUODENUM	-	-	-	-	-	-	-	-	-	-
JEJUNUM	-	-	-	-	-	-	-	-	-	-
ILEUM	-	-	-	-	-	-	-	-	-	-
PEYER'S PATCHES	-	-	-	+	-	-	-	-	-	-
- Mineraliz., focal	.	.	.	1.
CECUM	-	-	-	-	-	-	-	-	-	-
COLON	-	-	-	-	-	-	-	-	-	-
RECTUM	-	-	-	-	-	-	-	-	-	-
LIVER	+	+	+	+	+	-	-	-	-	-
- Hemopoietic cells	.	1.
- Inflamm. cell foci	1.	1.	1.	1.	1.
- Inflamm. peribiliary	.	.	1.

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TEST ITEM :
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SPONSOR : Cheil Industries Inc.

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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER :

	21	22	23	24	25	26	27	28	29	30
	MK0	MK0	MK0	MK0	MK0	MR1	MR1	MR1	MR1	MR1
KIDNEYS	-	+	+	+	-	'	'	'	'	'
- Dilatation, tubular	.	1.	.	.	.					
- Basophilia, tubular	.	.	1.	1.	.					
.....										
URINARY BLADDER	-	-	-	-	-	'	'	'	'	'
.....										
TESTES	-	-	-	-	-	'	'	'	'	'
.....										
EPIDIDYMIDES	-	-	-	-	-	'	'	'	'	'
.....										
PROSTATE GLAND	-	-	-	-	-	'	'	'	'	'
.....										
COAGULATING GLANDS	-	'	-	-	-	'	'	'	'	'
.....										
SEMINAL VESICLES	-	-	-	-	-	'	'	'	'	'
.....										
PITUITARY GLAND	-	-	-	-	+	'	'	'	'	'
- Cyst	P.					
.....										
THYROID GLAND	-	-	-	-	-	'	'	'	'	'
.....										
PARATHYROID GLANDS	-	-	-	-	(-	'	'	'	'	'
.....										
ADRENAL GLANDS	-	+	-	-	-	'	'	'	'	'
- Vacuolation, z.fas.	.	1.	.	.	.					
.....										
SPLEEN	+	+	-	+	+	'	'	'	'	'
- Hemopoietic foci	1.	1.	.	1.	1.					
.....										
BONE MARROW - STERN.	-	-	-	-	-	'	'	'	'	'
.....										
BONE MARROW, FEMUR	-	-	-	-	-	'	'	'	'	'
.....										
THYMUS	-	-	-	-	+	'	'	'	'	'
- Lymphoid atrophy	1.					
.....										
MESENT. LYMPH NODE	-	-	-	-	-	'	'	'	'	'
.....										

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TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
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TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER :

	21	22	23	24	25	26	27	28	29	30
	MK0	MK0	MK0	MK0	MK0	MR1	MR1	MR1	MR1	MR1
MANDIB. LYMPH NODES	-	-	+	-	-	'	'	'	'	'
- Plasmacytosis	.	.	1.	.	.					
SKELETAL MUSCLE	-	-	-	-	-	'	'	'	'	'
BONE - STERNUM	-	-	-	-	-	'	'	'	'	'
JOINTS, KNEE	-	-	-	-	-	'	'	'	'	'
EYES	-	-	-	-	-	'	'	'	'	'
FEMUR	-	-	-	-	-	'	'	'	'	'

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 27/ 53
TOX : 490627

TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER :

	51	52	53	54	55	56	57	58	59	60
	FK0	FK0	FK0	FK0	FK0	FR1	FR1	FR1	FR1	FR1
BRAIN	-	-	-	-	-	1	1	1	1	1
SPINAL CORD - CERV.	-	-	-	-	-	1	1	1	1	1
SPINAL CORD - THOR.	-	-	-	-	-	1	1	1	1	1
SPINAL CORD - LUMBAR	-	-	-	-	-	1	1	1	1	1
SCIATIC NERVE	-	-	+	-	-	1	1	1	1	1
- Myelin fragmentation	.	.	1.	.	.					
HEART	-	+	-	-	+	1	1	1	1	1
- Inflamm. lymphocytic	.	1.	.	.	1.					
TRACHEA	-	-	-	-	-	1	1	1	1	1
LUNG	+	-	+	+	+	1	1	1	1	1
- Lymphoid hyperplasia	1.	.	1.	1.	1.					
STOMACH	-	-	-	-	-	1	1	1	1	1
DUODENUM	-	-	-	-	-	1	1	1	1	1
JEJUNUM	-	-	-	-	-	1	1	1	1	1
ILEUM	-	-	-	-	-	1	1	1	1	1
PEYER'S PATCHES	-	-	-	-	-	1	1	1	1	1
CECUM	-	-	-	-	-	1	1	1	1	1
COLON	-	-	-	-	-	1	1	1	1	1
RECTUM	-	-	-	-	-	1	1	1	1	1
LIVER	+	+	+	+	+	1	1	1	1	1
- Hemopoietic cells	.	1.	.	.	.					
- Inflamm. cell foci	1.	1.	1.	1.	1.					

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 28/ 53
TOX : 490627

TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)
DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER :

	51	52	53	54	55	56	57	58	59	60
	FK0	FK0	FK0	FK0	FK0	FR1	FR1	FR1	FR1	FR1
KIDNEYS	-	-	+	+	-					
- Hyaline cast(s)	.	.	(1.	.	.					
- Basophilia, tubular	.	.	.	(1.	.					
URINARY BLADDER	-	-	-	-	-					
OVARIES	-	-	-	-	-					
UTERUS	-	-	-	-	+G			+G		+G
- Estrus epithelium	P.			P.		P.
CERVIX	-	-	-	-	-					
VAGINA	-	-	-	-	-					
PITUITARY GLAND	-	-	-	-	-					
THYROID GLAND	-	-	-	-	-					
PARATHYROID GLANDS	(-	-	(-	-	(-					
ADRENAL GLANDS	-	-	-	-	-					
SPLEEN	+	+	+	+	+					
- Hemopoietic foci	.	1.	.	.	1.					
- Hemosiderin pigment	1.	1.	1.	1.	1.					
BONE MARROW - STERN.	-	-	-	-	-					
BONE MARROW, FEMUR	-	-	-	-	-					
THYMUS	-	-	-	-	-					
MESENT. LYMPH NODE	-	-	-	-	-					
MANDIB. LYMPH NODES	-	-	-	-	-					
SKELETAL MUSCLE	-	-	-	-	-					

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 29/ 53
TOX : 490627

TEST ITEM : ☐ ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)

DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER :

	51	52	53	54	55	56	57	58	59	60
	FK0	FK0	FK0	FK0	FK0	FR1	FR1	FR1	FR1	FR1
BONE - STERNUM	-	-	-	-	-					
JOINTS, KNEE	-	-	-	-	-					
EYES	-	-	-	-	-					
FEMUR	-	-	-	-	-					

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 30/ 53
TOX : 490627

TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

ANIMAL HEADING DATA

DOSE GROUP : 01, CONTROL

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY	TEST DAYS	FIRST AND LAST DAY UNDER TEST	DATE OF NECROPSY
1	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
2	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
3	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
4	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
5	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
6	M	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
7	M	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
8	M	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
9	M	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
10	M	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
31	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
32	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
33	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
34	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
35	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
36	F	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
37	F	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
38	F	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
39	F	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09
40	F	R1 R1	28	13-MAR-09 09-APR-09	24-APR-09

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 31/ 53
TOX : 490627

TEST ITEM	: []	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

MALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 1

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG:

-Mineralization, vascular, grade 1

-Osseous metaplasia, focal, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Hyaline droplets, bilateral, grade 1

-Basophilia, tubular, corticomedullary, bilateral, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

MANDIBULAR LYMPH NODES:

-Macrophage foci, unilateral, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 2

* NECROPSY FINDINGS

SEMINAL VESICLES:

RIGHT SIDE: REDUCED IN SIZE.

NO OTHER NECROPSY OBSERVATIONS NOTED

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 32/ 53
TOX : 490627

TEST ITEM	:		PATHOL. NO.:	20907 BRH
TEST SYSTEM	:	RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	:	Cheil Industries Inc.	PathData@System	V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

MALE

CONT./FF. ANIMAL NO. : 2
.....

* MICROSCOPIC FINDINGS

SCIATIC NERVE:

-Myelin fragmentation, focal/multifocal, bilateral, grade 1

HEART:

-Inflammation lymphocytic, right ventricular wall, grade 1

LUNG:

-Lymphoid (BALT) hyperplasia, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Cyst(s), unilateral, grade 2

-Inflammation, interstitial lymphocytic, unilateral, grade 1

-Basophilia, tubular, corticomedullary, unilateral, grade 1

PROSTATE GLAND:

-Inflammation lymphocytic, grade 1

SEMINAL VESICLES:

-Reduced contents, unilateral, grade 1

This finding corresponds to necropsy observation no: 01.

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 3
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 33/ 53
TOX : 490627

TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.
PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

MALE

CONT./FF. ANIMAL NO. : 3
.....

* MICROSCOPIC FINDINGS

LUNG:

-Mineralization, vascular, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

PROSTATE GLAND:

-Inflammation lymphocytic, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 4
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SCIATIC NERVE:

-Myelin fragmentation, focal/multifocal, bilateral, grade 1

LUNG:

-Lymphoid (BALt) hyperplasia, grade 2

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:


-Inflammation, interstitial lymphocytic, unilateral, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

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PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 34/ 53
TOX : 490627

TEST ITEM	: 	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

MALE

CONT./FF. ANIMAL NO. : 4
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 5
.....

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Hyaline droplets, bilateral, grade 1

-Basophilia, tubular, corticomedullary, unilateral, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 2

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 35/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

FEMALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 31

* NECROPSY FINDINGS

UTERUS:

CONTAINS FLUID.

NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

UTERUS:

-Proestrus epithelium

This finding corresponds to necropsy observation no: 01.

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

-Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 32

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 36/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

FEMALE

CONT./FF. ANIMAL NO. : 32

* MICROSCOPIC FINDINGS

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Basophilia, tubular, corticomedullary, unilateral, grade 1

PARATHYROID GLANDS:

Only one of paired organs examined/present

SPLEEN:

-Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 33

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG:

-Alveolar inflammation, lymphocytic, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 37/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2dl

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

FEMALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 34

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG:

-Mineralization, vascular, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

-Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 35

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SCIATIC NERVE:

-Myelin fragmentation, focal/multifocal, bilateral, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 38/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

FEMALE

CONT./FF. ANIMAL NO. : 35

SPLEEN:

- Hemopoietic foci, primarily erythropoiesis, grade 1
- Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: R1

DAYS ON TEST : 28

* ANIMAL NO. : 36

* NECROPSY FINDINGS

CLITORAL GLANDS:

LEFT SIDE: DISCOLOURATION, DARK RED.

NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

CLITORAL GLANDS:

- Dilated gland with contents, unilateral, grade 2

This finding corresponds to necropsy observation no: 01.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 39/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

FEMALE

* STATE AT NECROPSY: R1

DAYS ON TEST : 28

* ANIMAL NO. : 38

* NECROPSY FINDINGS

UTERUS:

CONTAINS FLUID.

NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

UTERUS:

-Proestrus epithelium

This finding corresponds to necropsy observation no: 01.

* STATE AT NECROPSY: R1

DAYS ON TEST : 28

* ANIMAL NO. : 39

* NECROPSY FINDINGS

CLITORAL GLANDS:

RIGHT SIDE: FOCUS/FOCI, ISOLATED, TAN.

NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

CLITORAL GLANDS:

-Dilated gland with contents, unilateral, grade 1

This finding corresponds to necropsy observation no: 01.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 40/ 53
TOX : 490627

TEST ITEM : C-7
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 01, CONTROL

FEMALE

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 41/ 53
TOX : 490627

TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

ANIMAL HEADING DATA

DOSE GROUP : 02, 30 MG/KG

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY	TEST DAYS	FIRST AND LAST DAY UNDER TEST	DATE OF NECROPSY
11	M	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
12	M	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
13	M	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
14	M	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
15	M	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
41	F	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
42	F	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
43	F	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
44	F	KO KO	28	13-MAR-09 09-APR-09	10-APR-09
45	F	KO KO	28	13-MAR-09 09-APR-09	10-APR-09

- ALL ANIMALS WITHOUT PATHOLOGIC FINDINGS. -

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

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TOX : 490627

TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

ANIMAL HEADING DATA

DOSE GROUP : 03, 300 MG/KG

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY	TEST DAYS	FIRST AND LAST DAY UNDER TEST	DATE OF NECROPSY
16	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
17	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
18	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
19	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
20	M	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
46	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
47	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
48	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
49	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09
50	F	K0 K0	28	13-MAR-09 09-APR-09	10-APR-09

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 43/ 53
TOX : 490627TEST ITEM : []
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 03, 300 MG/KG

FEMALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 49

* NECROPSY FINDINGS

UTERUS:

CONTAINS FLUID.

NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

UTERUS:

-Estrus epithelium

This finding corresponds to necropsy observation no: 01.

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

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PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATA

PAGE : 44/ 53
TOX : 490627

TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.

PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

ANIMAL HEADING DATA

DOSE GROUP : 04, 1000 MG/KG

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY	TEST DAYS	FIRST AND LAST DAY UNDER TEST	DATE OF NECROPSY
21	M	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
22	M	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
23	M	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
24	M	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
25	M	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
26	M	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
27	M	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
28	M	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
29	M	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
30	M	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
51	F	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
52	F	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
53	F	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
54	F	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
55	F	K0	K0	28	13-MAR-09 09-APR-09 10-APR-09
56	F	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
57	F	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
58	F	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
59	F	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09
60	F	R1	R1	28	13-MAR-09 09-APR-09 24-APR-09

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 45/ 53
TOX : 490627TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData©System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

MALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 21

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 22

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:

-Hemopoietic cell foci, grade 1

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Dilatation, tubular, bilateral, grade 1

ADRENAL GLANDS:

-Vacuolation, zona fasciculata, multifocal, bilateral, grade 1

445

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 46/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

MALE

CONT./FF. ANIMAL NO. : 22

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 23

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG:

-Alveolar inflammation, lymphocytic, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

-Inflammation peribiliary, lymphocytic, grade 1

KIDNEYS:

-Basophilia, tubular, corticomedullary, bilateral, grade 1

MANDIBULAR LYMPH NODES:

-Plasmacytosis, bilateral, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 47/ 53
TOX : 490627TEST ITEM : ☐
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

MALE

* STATE AT NECROPSY: KO

DAYS ON TEST : 28

* ANIMAL NO. : 24

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

PEYER'S PATCHES:

-Mineralization, focal, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Basophilia, tubular, corticomedullary, bilateral, grade 1

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: KO

DAYS ON TEST : 28

* ANIMAL NO. : 25

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

PITUITARY GLAND:

-Cyst, pars distalis

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 48/ 53
TOX : 490627

TEST ITEM	: <input type="checkbox"/>	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData@System	V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

MALE

CONT./FF. ANIMAL NO. : 25
.....

PARATHYROID GLANDS:

Only one of paired organs examined/present

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

THYMUS:

-Lymphoid atrophy (involution), grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 49/ 53
TOX : 490627

TEST ITEM	: []	PATHOL. NO.:	20907 BRH
TEST SYSTEM	: RAT, 28 day with rec., Gavage	DATE	: 26-MAY-09
SPONSOR	: Cheil Industries Inc.	PathData©System	V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

FEMALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 51

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG:

-Lymphoid (BALT) hyperplasia, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

PARATHYROID GLANDS:

Only one of paired organs examined/present

SPLEEN:

-Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 52

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

HEART:

-Inflammation lymphocytic, right ventricular wall, grade 1

LIVER:

-Hemopoietic cell foci, grade 1

-Inflammatory cell foci, lymphocytic, grade 1

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 50/ 53
TOX : 490627TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

FEMALE

CONT./FF. ANIMAL NO. : 52

SPLEEN:

- Hemopoietic foci, primarily erythropoiesis, grade 1
- Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 53

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

SCIATIC NERVE:

- Myelin fragmentation, focal/multifocal, bilateral, grade 1

LUNG:

- Lymphoid (BALT) hyperplasia, grade 1

LIVER:

- Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

- Hyaline cast(s), unilateral, grade 1

PARATHYROID GLANDS:

Only one of paired organs examined/present

SPLEEN:

- Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 51/ 53
TOX : 490627TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

FEMALE

* STATE AT NECROPSY: K0

DAYS ON TEST : 28

* ANIMAL NO. : 54

* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

* MICROSCOPIC FINDINGS

LUNG:

-Lymphoid (BALT) hyperplasia, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

KIDNEYS:

-Basophilia, tubular, corticomedullary, unilateral, grade 1

SPLEEN:

-Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: K0

DAYS ON TEST : 28


* ANIMAL NO. : 55

* NECROPSY FINDINGS

UTERUS:

CONTAINS FLUID.

NO OTHER NECROPSY OBSERVATIONS NOTED

PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 52/ 53
TOX : 490627TEST ITEM : 
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

FEMALE

CONT./FF. ANIMAL NO. : 55

* MICROSCOPIC FINDINGS

HEART:

-Inflammation lymphocytic, right ventricular wall, grade 1

LUNG:

-Lymphoid (BALT) hyperplasia, grade 1

LIVER:

-Inflammatory cell foci, lymphocytic, grade 1

UTERUS:

-Estrus epithelium

This finding corresponds to necropsy observation no: 01.

PARATHYROID GLANDS:

Only one of paired organs examined/present

SPLEEN:

-Hemopoietic foci, primarily erythropoiesis, grade 1

-Hemosiderin pigment, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

* STATE AT NECROPSY: R1

DAYS ON TEST : 28

* ANIMAL NO. : 58

* NECROPSY FINDINGS

UTERUS:

CONTAINS FLUID.

NO OTHER NECROPSY OBSERVATIONS NOTED

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PATHOLOGY REPORT
INDIVIDUAL ANIMAL DATAPAGE : 53/ 53
TOX : 490627TEST ITEM :
TEST SYSTEM : RAT, 28 day with rec., Gavage
SPONSOR : Cheil Industries Inc.PATHOL. NO.: 20907 BRH
DATE : 26-MAY-09
PathData@System V6.2d1

TEXT OF GROSS AND MICROSCOPIC FINDINGS

DOSE GROUP : 04, 1000 MG/KG

FEMALE

CONT./FF. ANIMAL NO. : 58

* MICROSCOPIC FINDINGS

UTERUS:

-Estrus epithelium

This finding corresponds to necropsy observation no: 01.

* STATE AT NECROPSY: R1

DAYS ON TEST : 28

* ANIMAL NO. : 60

* NECROPSY FINDINGS

UTERUS:

CONTAINS FLUID.

NO OTHER NECROPSY OBSERVATIONS NOTED

* MICROSCOPIC FINDINGS

UTERUS:


-Estrus epithelium

This finding corresponds to necropsy observation no: 01.

- ALL OTHER ANIMALS IN DOSE GROUP WITHOUT PATHOLOGICAL FINDINGS -

METI SUMMARY

Study Title

SUBACUTE 28-DAY ORAL TOXICITY WITH

BY DAILY GAVAGE IN THE RAT,
FOLLOWED BY A 14-DAY RECOVERY PERIOD

Author

F.M. van Otterdijk, M.Sc.

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

NOTOX Project 490627
NOTOX Substance 190683/B

Acute Toxicity Test or Preliminary Repeated Dose Toxicity Test

Test no.	Kind and term of test	Animal species	Number of animals per group	Route of administration	Dose levels (mg/kg bw)	NOEL* (mg/kg bw)	Testing institute
1	5-d range finding	Rat	3 females	Oral gavage	500 1000	1000	NOTOX

*. NOEL: No observed effect level

Twenty-Eight Day Repeated Dose Toxicity Test

Administration period of the test substance: from 13 March 2009 to 09 April 2009						
Animal species / strain:	Wistar rat CrI:WI(Han)		Number of animals per group			
Route of administration:	oral gavage		Males		Females	
Purity of test substance:	> 99%		10 (groups 1 + 4) 5 (groups 2 + 3)		10 (groups 1 + 4) 5 (groups 2 + 3)	
Parameter	Group 1 Control 0 [#]	Group 1 Recovery 0 [#]	Group 2 Low dose 30 [#]	Group 3 Mid dose 300 [#]	Group 4 High dose 1000 [#]	Group 4 Recovery 1000 [#]
Body weight	Normal	Normal	Same as control	Same as control	Same as control	Same as control
Food consumption	Normal	Normal	Same as control	Same as control	Same as control	Same as control
General appearance	Normal	Normal	Normal	Normal	Normal	Normal
Funct. observations	Normal	ND	Normal	Normal	Normal	ND
Haematology	Normal	Normal	Normal	1	1	2
Blood biochemistry	Normal	Normal	Normal	Normal	Normal	Normal
Macroscopy	Normal	Normal	Normal	Normal	Normal	Normal
Organ weights	Normal	Normal	Normal	3	3, 4	Normal
Histology	Normal	Normal	Normal	Normal	Normal	Normal
NOAEL*	1000 mg/kg body weight/day					
Change(-s) by which NOAEL is estimated	Not applicable					

* No observed adverse effect level:

The higher cholesterol levels for females at 300 and 1000 mg/kg/day at the end of the treatment period, and higher creatinine and potassium levels in females at 1000 mg/kg/day exceeded the range considered normal for rats of this age and strain. There were however no histopathological correlates and either these changes had recovered during the recovery period (cholesterol) or these were not apparent at the end of the treatment period (creatinine and potassium). For creatinine and potassium a similar or more pronounced change would be expected at the end of the treatment period.

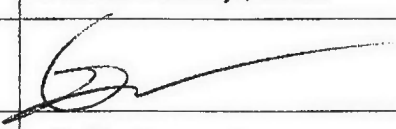
The slightly higher liver to body weight ratios of males and females at 300 and 1000 mg/kg/day, and higher thyroid to body weight ratio of females at 1000 mg/kg/day at the end of the treatment period occurred without histopathological correlates and changes were slight in nature. At the end of the recovery period, relative liver and thyroid weights were similar to control levels. Therefore, these changes were considered not to be of a toxicologically significant nature.

Dose level in mg/kg body weight/day.

ND Not determined; no treatment-related findings at the end of treatment.

1. Higher cholesterol levels (females).
2. Higher creatinine and potassium levels (females)
3. Slightly higher liver to body weight ratios (males and females).
4. Slightly higher thyroid to body weight ratio (females).

Others

Testing institute	Name	NOTOX B.V.
	Address	Hambakenwetering 7 5203 DL 's Hertogenbosch The Netherlands Tel.: +31(0)73 640 67 00 Fax: +31(0)73 640 67 99
Study Director	Name, Title	F.M. van Otterdijk, M.Sc.
	Signature	
	Date	27 May 2009
	Years of experience	10
NOTOX Project Number	490627	
Test period	from 04 March 2009 to 24 April 2009	

ACUTE TOXICITY TO RAINBOW TROUT
(*Oncorhynchus mykiss*)

PROJECT NUMBER: 2737/0001

AUTHOR: S L Priestly

STUDY SPONSOR:

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KOREA

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Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

2737-0001.doc/L

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

13 November 2008	Standard Test Method Compliance Audit
19 May 2009	Test Material Preparation
05 May 2009	Test System Preparation
05 May 2009	Exposure
01 May 2009	Assessment of Response
08, 12 May 2009	Chemical Analysis
§ 25 June 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§ Evaluation specific to this study	

..... G. Wren
For the Quality Assurance Unit*

DATE: 30 JUN 2009

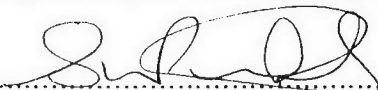
***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.



Date: 29 JUN 2009

S L Priestly BSc
Study Director

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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ACUTE TOXICITY TO RAINBOW TROUT
(*Oncorhynchus mykiss*)

SUMMARY

Introduction. A study was performed to assess the acute toxicity of the test material to rainbow trout (*Oncorhynchus mykiss*). The method followed that described in the OECD Guidelines for Testing of Chemicals (1992) No 203, "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Regulation (EC) No. 440/2008.

Methods. Information provided by the Sponsor indicated that the test material was insoluble in water. Pre-study solubility work conducted indicated that it was not possible to obtain a testable solution of the test material using traditional methods of preparation e.g. ultrasonication and high shear mixing.

A pre-study media preparation trial indicated that a dissolved test material concentration of approximately 0.0041 mg/l was obtained from a saturated solution method of preparation followed by centrifugation indicating this to be the limit of water solubility of this material under test conditions.

As it was impractical to centrifuge large volumes of test media prior to exposure it was considered appropriate to prepare the test media using a preliminary solution in tetrahydrofuran and analyze samples of uncentrifuged and centrifuged test media over the duration of the definitive test. This approach allowed the determination of the total amount of test material present (uncentrifuged test samples) and the amount of dissolved test material within the test system (centrifuged test samples) and hence bioavailable to the test organisms.

Following a preliminary range-finding test fish were exposed, in two groups of seven, to an aqueous dispersion of the test material, at a single nominal concentration of 0.0040 mg/l for a period of 96 hours at a temperature of approximately 14°C under semi-static test conditions. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

Chemical analysis of the centrifuged test preparations in the definitive test showed measured concentrations to be lower than 0.0040 mg/l, this was considered to be due to slight differences in media and/or sampling techniques used to remove the supernatant after centrifugation between the media preparation trial and the definitive test. It was therefore considered appropriate to express the concentrations in the definitive test in terms of the time-weighted mean measured concentration of 0.0034 mg/l.

Results. The results of the solubility trials indicated that at a test concentration of 0.0040 mg/l prepared using a preliminary solution in auxiliary solvent to spike the test medium, a significant proportion of undissolved/dispersed test material would be present. Samples were therefore analysed untreated and after centrifugation (40000 g, 30 minutes) in order to give an indication of the dissolved and hence bioavailable test material concentration.

Analysis of the fresh media at 0, 24, 48 and 72 hours showed the measured concentrations to range from 0.00545 to 0.00608 mg/l for the untreated samples. Analysis of the untreated old media samples at 24, 48, 72 and 96 hours showed a slight decline in measured concentrations of 0.00424 to 0.00517 mg/l. The high results obtained for the untreated test media were considered to be due to the presence of undissolved test material in the test media.

Analysis of the centrifuged fresh media at 0, 24, 48 and 72 hours showed the measured concentrations to range from 0.00326 to 0.00471 mg/l. Analysis of the centrifuged old media samples at 24, 48, 72 and 96 hours showed a slight decline in measured concentrations of 0.00229 to 0.00346 mg/l.

Given this decline in measured test concentrations it was considered justifiable to base the results on the time-weighted mean measured test concentrations of the centrifuged test media to give a "worst case" analysis of the data. The 96-Hour LC₅₀ based on the time-weighted mean measured, and hence bioavailable test material concentration, was greater than 0.0034 mg/l and correspondingly the No Observed Effect Concentration was 0.0034 mg/l.

The time-weighted mean measured test concentration of 0.0034 mg/l was the highest attainable test concentration due to the limited solubility of the test material in water and auxiliary solvent, and having due regard for the amount of auxiliary solvent permitted in the test under the OECD Guidelines.

ACUTE TOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*)

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of the test material to rainbow trout. The method followed the recommendations of the OECD Guidelines for Testing of Chemicals (1992) No 203 "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Regulation (EC) No. 440/2008.

Rainbow trout is a freshwater fish representative of a wide variety of natural habitats, and can therefore be considered as an important non-target organism in freshwater ecosystems.

The study was conducted between 05 May 2009 and 19 June 2009

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	
Description	:	white powder
Batch number	:	20090105
Date received	:	23 January 2009
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor. A Certificate of Analysis for the test material supplied by the Sponsor is given in Appendix 1.

3. METHODS

3.1 Test Species

The test was carried out using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were obtained from Brow Well Fisheries Limited, Hebden, near Skipton, Yorkshire, UK and

maintained in-house since 20 May 2009. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 3 June 2009 to 15 June 2009. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle with 20 minute dawn and dusk transition periods.

The water temperature was controlled at approximately 14°C with a dissolved oxygen content of greater than or equal to 9.8 mg O₂/l. These parameters were recorded daily. The stock fish were fed commercial trout pellets which was discontinued 24 hours prior to the start of the definitive test. There was zero mortality in the 7 days prior to the start of the test and the fish had a mean standard length of 5.0 cm (sd = 0.1) and a mean weight of 1.71 g (sd = 0.08) at the end of the definitive test. Based on the mean weight value this gave a loading rate of 0.60 g bodyweight/litre.

The diet and diluent water are considered not to contain any contaminant that would affect the integrity and outcome of the study.

3.2 Test Water

The test water used for both the range-finding and definitive tests was the same as that used to maintain the stock fish.

Laboratory tap water was dechlorinated by passage through an activated carbon filter (Purite Series 500) and partly softened (Elga Nimbus 1248D Duplex Water Softener) giving water with a total hardness of approximately 140 mg/l as CaCO₃. After dechlorination and softening the water was passed through a series of computer controlled plate heat exchangers to achieve the required temperature. Typical water quality characteristics for the tap water as supplied, prior to dechlorination and softening, are given in Appendix 2.

3.3 Procedure

3.3.1 Pre-study media preparation trial

Information provided by the Sponsor indicated that the test material was insoluble in water. Pre-study solubility work conducted indicated that it was not possible to obtain a testable solution of the test material using traditional methods of preparation e.g. ultrasonication and high shear mixing.

Pre-study solubility work conducted indicated that the test material was practically insoluble in water using traditional methods of preparation e.g. ultrasonication and high shear mixing.

Based on this information the test material was categorised as being a 'difficult substance' as defined by the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 2000). Therefore a media preparation trial was conducted in order to determine the solubility of the test material under test conditions.

An amount of test material (550 mg) was dispersed, in duplicate, in 11 litres of reconstituted water with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 21°C for periods of 24 or 48 hours. After stirring samples were taken for chemical analysis after the following pre-treatments:

- Centrifugation at 10000 *g* for 30 minutes
- Centrifugation at 40000 *g* for 30 minutes
- Filtration through a 0.2 µm Sartorius Sartopore filter (approximately 500 ml discarded in order to pre-condition the filter)
- Filtration through a 0.2 µm Sartorius Sartopore filter (approximately 1 litre discarded in order to pre-condition the filter)

3.3.2 Range-finding test

The test concentration to be used in the definitive test was determined by a preliminary range-finding test.

In the range-finding test fish were exposed to a single nominal test concentration of 0.0040 mg/l as the results from the Acute Toxicity to *Daphnia magna* test (Harlan Laboratories Ltd Project Number: 2737/0002) indicated that toxicity was not expected at this level. The test material was prepared using a preliminary solution in tetrahydrofuran.

An amount of test material (100 mg) was dissolved in tetrahydrofuran and the volume adjusted to 25 ml to give a 100 mg/25 ml solvent stock solution. Serial dilutions were performed from this to give the 0.40 mg/10 ml solvent stock solution. An aliquot (2.0 ml) of this was dispersed in 20 litres of dechlorinated tap water and stirred with a flat bladed mixer for approximately 1 minute to give the 0.0040 mg/l test concentration.

Each of the stock solutions were inverted several times to ensure adequate mixing and homogeneity.

In the range-finding test 3 fish were added to each 20 litre test and control vessel and maintained at approximately 14°C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods for a period of 96 hours under static test conditions.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of tetrahydrofuran.

Each vessel was covered to reduce evaporation. After 3, 6, 24, 48, 72 and 96 hours any mortalities or sub-lethal effects of exposure were determined by visual inspection of the test fish.

3.3.3 Definitive test

Based on the results of the range-finding test a "Limit test" was conducted at a concentration of 0.0040 mg/l to confirm that at the highest attainable test concentration of 0.0040 mg/l, no mortalities or sub-lethal effects of exposure were observed.

Chemical analysis of the centrifuged test preparations in the definitive test showed measured concentrations to be lower than 0.0040 mg/l, this was considered to be due to slight differences in media and/or sampling techniques used to remove the supernatant after centrifugation between the media preparation trial and the definitive test. It was therefore considered appropriate to express the concentrations in the definitive test in terms of the time-weighted mean measured concentration of 0.0034 mg/l.

3.3.3.1 Experimental Preparation

For the purpose of the definitive test the test material was prepared using a preliminary solution in tetrahydrofuran.

An amount of test material (100 mg) was dissolved in tetrahydrofuran and the volume adjusted to 25 ml to give a 100 mg/25 ml solvent stock solution. Serial dilutions were prepared from this to give a 0.40 mg/10 ml solvent stock solution. An aliquot (2.0 ml) of the 0.40 mg/25 ml solvent stock solution was dispersed in dechlorinated tap water and

stirred with a flat bladed mixer for approximately 1 minute to give the required test concentration. This method of preparation was conducted in duplicate to give replicates R_1 and R_2 .

Each of the stock solutions were inverted several times to ensure adequate mixing and homogeneity.

The concentration and stability of the test material in the centrifuged and uncentrifuged samples were verified by chemical analysis at 0 (fresh media), 24, 48, 72 (old and fresh media) and 96 hours (old media) (see Appendix 3).

3.3.3.2 Exposure conditions

As in the range-finding test 20 litre glass exposure vessels were used for each test concentration. At the start of the test 7 fish were placed in each test vessel at random, in the test preparations. The test vessels were then covered to reduce evaporation and maintained at approximately 14°C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods for a period of 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.

The control and solvent control groups were maintained under identical conditions but not exposed to the test material. The solvent control group was exposed to 100 µl/l of tetrahydrofuran. Data from the control group was shared with similar concurrent studies.

A semi-static test regime was employed in the test involving a daily renewal of the test preparations to ensure that the concentrations of the test material remained near nominal and to prevent the build up of nitrogenous waste products.

Any mortalities and sub-lethal effects of exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death were taken to be the absence of both respiratory movement and response to physical stimulation.

3.3.3.3 Physico-chemical measurements

The water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the test. The measurements at 0 hours, and after each test media renewal at 24, 48 and 72 hours, represent those of the freshly prepared test preparations while the

measurements taken prior to each test media renewal, and on termination of the test after 96 hours, represent those of the used or 24-Hour old test preparations. The pH and the dissolved oxygen concentration was measured using a WTW pH/Oxi 340I pH and dissolved oxygen meter and the temperature was measured using Hanna Instruments HI 93510 digital thermometer.

3.3.3.4 *Verification of test concentrations*

Water samples were taken from the solvent control and each replicate test vessel at 0 (fresh media), 24, 48, 72 (old and fresh media) and 96 hours (old media) for quantitative analysis.

Two samples of the solvent control and each surviving test group replicate were taken at each occasion. One sample was analysed untreated and one sample after centrifugation (40000 g for approximately 30 minutes). Further samples (in duplicate) were taken and stored at approximately -20°C for further analysis, if necessary.

The method of analysis, stability, recovery and test preparation analyses are described in Appendix 3.

3.3.3.5 *Evaluation of data*

An estimate of the LC₅₀ values was given by inspection of the mortality data.

The time-weighted mean measured test concentrations of the centrifuged samples using the mean of the measured test concentrations of replicates R₁ and R₂ were calculated as follows:

$$TWM = \frac{\text{Total area}}{\text{Total number of days of the test}}$$

$$\text{where Total area} = \frac{C_0 - C_1}{\ln(C_0) - \ln(C_1)} \times \text{days}$$

- TWM = time-weighted mean measured test concentration (mg/l)
 C₀ = measured concentration at the start of each renewal period (mg/l)
 C₁ = measured concentration at the end of each renewal period (mg/l)
 Days = number of days in the renewal period

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Pre-Study Media Preparation Trial

The results obtained from the pre-study media preparation trial conducted (see Appendix 3) indicated that the test material was adsorbing to the filter matrices. There was no significant increase in the dissolved test material concentration obtained when the preparation period of the saturated solution was extended to 48 hours.

As it was impractical to centrifuge large volumes of test media prior to exposure it was considered appropriate to prepare the test media using a preliminary solution in tetrahydrofuran and analyze samples of uncentrifuged and centrifuged test media over the duration of the definitive test. This approach allowed the determination of the total amount of test material present (uncentrifuged test samples) and the amount of dissolved test material within the test system (centrifuged test samples) and hence bioavailable to the test organisms.

5.2 Range-finding Test

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding test are given in Table 1. A single mortality was observed after 48 hours, this was considered to be due to natural causes as no further mortalities or adverse effects due to exposure were observed. There were no sub-lethal effects of exposure during the range-finding test.

Based on this information, a single nominal test concentration, in duplicate, of 0.0040 mg/l was selected for the definitive test. This experimental design conforms to a "Limit test" to confirm that at the highest attainable test concentration of 0.0040 mg/l, no mortalities or sub-lethal effects of exposure were observed.

5.3 Definitive Test

5.3.1 Verification of test concentrations

Analysis of the fresh media at 0, 24, 48 and 72 hours (see Appendix 3) showed the measured concentrations to range from 0.00545 to 0.00608 mg/l for the untreated samples. Analysis of the untreated old media samples at 24, 48, 72 and 96 hours showed a slight decline in measured concentrations of 0.00424 to 0.00517 mg/l. The

high results obtained for the untreated test media were considered to be due to the presence of undissolved test material in the test media.

Analysis of the centrifuged fresh media at 0, 24, 48 and 72 hours (see Appendix 3) showed the measured concentrations to range from 0.00326 to 0.00471 mg/l. Analysis of the centrifuged old media samples at 24, 48, 72 and 96 hours showed a slight decline in measured concentrations of 0.00229 to 0.00346 mg/l.

A Study to Determine the Partition Coefficient of the test material (Harlan Laboratories Ltd Project Number: 2737/0004) gave $\log_{10} P_{ow}$ value of greater than 6.5. Therefore it was considered that the decline in measured concentration over each 24-Hour dosing period was due to possible bioaccumulation.

Current regulatory advice is that in cases where a decline in measured concentrations is observed, time-weighted mean measured concentrations should be used for calculating LC_{50} values. It was therefore considered justifiable to base the results on the time-weighted mean measured test concentrations in order to give a "worst case" analysis of the data. The time-weighted mean measured test concentration was determined to be 0.034 mg/l.

5.3.2 Mortality data

Cumulative mortality data from the exposure of rainbow trout to the test material during the definitive test are given in Table 2.

There were no mortalities in 14 fish exposed to a time-weighted mean measured test concentration of 0.0034 mg/l for a period of 96 hours. Inspection of the mortality data gave the following results:

Time (h)	LC_{50} (mg/l)	95% Confidence limits (mg/l)
3	> 0.0034	-
6	> 0.0034	-
24	> 0.0034	-
48	> 0.0034	-
72	> 0.0034	-
96	> 0.0034	-

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The results of the definitive test showed the highest test concentration resulting in 0% mortality to be greater than or equal to 0.0034 mg/l, the lowest test concentration resulting in 100% mortality to be greater than 0.0034 mg/l and the No Observed Effect Concentration (NOEC) to be 0.0034 mg/l. The No Observed Effect Concentration is based upon zero mortalities and the absence of any sub-lethal effects of exposure at this concentration (Section 5.3.3).

The time-weighted mean measured test concentration of 0.0034 mg/l was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water and having due regard to the amount of auxiliary solvent permitted in the study under the OECD Guidelines. Other various recognised auxiliary solvents were used during preliminary solubility work, however, tetrahydrofuran was found to give the best testable dispersion of the test material in water.

5.3.3 Sub-lethal effects

There were no sub-lethal effects of exposure observed in 14 fish exposed to a time-weighted mean measured test concentration of 0.0034 mg/l for a period of 96 hours.

5.3.4 Observations on test material solubility

The test media preparations were observed to be clear, colourless solutions throughout the duration of the test.

5.3.5 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix 4. Temperature was maintained at approximately 14°C throughout the test, while there were no treatment related differences for oxygen concentration or pH.

The pH of the control and solvent control groups was observed to vary between 7.6 and 8.0. This variation was considered not to affect the validity or integrity of the test given that no mortalities or adverse reactions to exposure were observed in the control groups and the Test Guideline states that the pH should not vary by more than 1 unit.

6. CONCLUSION

Based on the time-weighted mean measured test concentrations of the centrifuged test media the acute toxicity of the test material to rainbow trout gave a 96-Hour LC₅₀ value of greater than 0.0034 mg/l. The No Observed Effect Concentration was 0.0034 mg/l.

7. REFERENCES

Environment Directorate, Organisation for Economic Co-operation and Development (OECD) (2000) Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures.

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Monograph No. 26 (1996) Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances.

ACUTE TOXICITY TO RAINBOW TROUT

Table 1 Cumulative Mortality Data in the Range-finding Test

Nominal Concentration (mg/l)	Cumulative Mortality (Initial Population = 3)					
	3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Solvent Control	0	0	0	0	0	0
0.0040	0	0	0	1	1	1

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Table 2 Cumulative Mortality Data in the Definitive Test

Time-weighted mean Measured Test Concentration (mg/l)	Cumulative Mortality (Initial Population = 7)						% Mortality
	3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours	96 Hours
Control	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0
0.0034 R ₁	0	0	0	0	0	0	0
0.0034 R ₂	0	0	0	0	0	0	0

R₁ and R₂ = Replicates 1 and 2

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Appendix 1 Certificate of Analysis

Certificate of Analysis

1. TEST MATERIAL IDENTIFICATION

- 1) PRODUCT NAME : []
2) TEST MATERIAL(LOT No.) : 20090105
3) QUANTITY : 100g
4) CHEMICAL NAME : []
5) COMPOSITIONS OF CHEMICAL []

- 6) PURITY : >99%
7) APPEARANCE : White Powder
8) MOLECULAR WEIGHT []
9) MOLECULAR FORMULA : []

2. SOLUBILITY :

- 1) INSOLUBLE : H₂O
2) SOLUBLE : Toluene(Partially)

3. STORAGE :

- 1) Storage temperature : Room Temperature
2) Expiry date : Jan. 08, 2011

We hereby certify that the data stated here above are true and correct

Company(Manufacturer) : CHEIL INDUSTRIAL INC.

Address : (437-711) 332-2, Gocheon-dong, Uiwang-si, Gyeonggi-do, Korea

Date : Jan. 09, 2009

Name of person in charge

Sunghee Ahn 

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 Typical Water Quality Characteristics

WATER SUPPLY ZONE ZDB13 ALVASTON								
REPORTING PERIOD: 01/01/2007 - 31/12/2007								
PARAMETER	ANALYSIS UNITS	NO. SAMPLES TAKEN	CONCENTRATION OR VALUE (ALL SAMPLES)			PCV*	SAMPLES CONTRAVENING PCV	
			MINIMUM	AVERAGE	MAXIMUM		NUMBER	PERCENT
1,2 Dichloroethane	µg/l	8	<0.080	<0.080	<0.080	3	0	0.00
2, 3, 6 - TBA	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
2, 4 - DB	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
2, 4, 5, - TCPA	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
2,4-D	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Aldrin	µg/l	8	<0.001	<0.001	<0.001	0.03	0	0.00
Alpha-HCH	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Aluminium	µg/l	52	<11.000	<12.692	29.000	200	0	0.00
Ametryn	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Antimony	µg/l	8	<0.120	<0.529	1.200	5	0	0.00
Arsenic	µg/l	8	<0.370	<0.370	<0.370	10	0	0.00
Atrazine	µg/l	8	<0.001	<0.001	0.001	0.1	0	0.00
Benazolin	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Bentazone	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Benzene	µg/l	8	<0.060	<0.060	<0.060	1	0	0.00
Benzo 3,4 Pyrene	µg/l	8	<0.001	<0.001	<0.001	0.01	0	0.00
Beta - HCH	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Boron	mg/l	8	0.021	0.034	0.052	1	0	0.00
Bromacil	µg/l	8	<0.012	<0.012	<0.012	0.1	0	0.00
Bromate	µg/l	8	<0.600	<0.600	<0.600	10	0	0.00
Bromoxynil	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Cadmium	µg/l	8	<0.060	<0.111	0.150	5	0	0.00
Captan	µg/l	8	<0.007	<0.007	<0.007	0.1	0	0.00
Carbenazim	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Carbetamide	µg/l	8	<0.002	<0.003	<0.008	0.1	0	0.00
Chlordane A	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Chlorothalonil	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
Chlorotoluron	µg/l	8	<0.002	<0.002	<0.004	0.1	0	0.00
Chromium	µg/l	8	<0.700	<0.700	<0.700	50	0	0.00
Cis - Permethrin	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Clopyralid	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Colour	mg/l Pt/Co	26	<0.400	<0.408	0.600	20	0	0.00
Copper	mg/l	8	<0.003	<0.008	0.027	2	0	0.00
Cyanazine	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Cyanide	µg/l	8	<0.400	<0.625	0.700	50	0	0.00
Cyfluthrin	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
Cypermethrin	µg/l	8	<0.004	<0.004	<0.004	0.1	0	0.00
D.D.E - Ortho Para	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
D.D.E - Para Para	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
D.D.T - Ortho Para	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
D.D.T - Para Para	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Delta - HCH	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Deltamethrin	µg/l	8	<0.005	<0.005	<0.005	0.1	0	0.00
Dicamba	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Dichlobenil	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Dichloprop	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Dieldrin	µg/l	8	<0.002	<0.002	<0.002	0.03	0	0.00
Diflufenican	µg/l	8	<0.004	<0.004	<0.004	0.1	0	0.00
Diuron	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
E. coli	No. / 100 ml	180	0.000	0.000	0.000	0	0	0.00
Endosulfan A	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Endosulfan B	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Endrin	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Enterococci	No. / 100 ml	8	0.000	0.000	0.000	0	0	0.00
EPTC	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Ethofumesate	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Fenoprop	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Fenpropidin	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00

*PCV Prescribed concentration or value for mandatory parameters and specified limit or value for non-mandatory parameters (indicators and disinfectants).

*1 No numerical limit applies.

*2 Guidance levels only. Monitored for the calculation of Total Indicative Dose, which is the regulatory parameter. No samples exceeded the TID in 2007.

*3 The formula for the calculated Nitrate/Nitrite level is [Nitrate]/50 + [Nitrite]/3. This number has no units.

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Typical Water Quality Characteristics

WATER SUPPLY ZONE ZDB13 ALVASTON								
REPORTING PERIOD: 01/01/2007 - 31/12/2007								
PARAMETER	ANALYSIS UNITS	NO. SAMPLES TAKEN	CONCENTRATION OR VALUE (ALL SAMPLES)			PCV*	SAMPLES CONTRAVENING PCV	
			MINIMUM	AVERAGE	MAXIMUM		NUMBER	PERCENT
Fenpropimorph	µg/l	8	<0.006	<0.006	<0.006	0.1	0	0.00
Fenvalerate	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
Fluoride	mg/l	8	0.310	0.351	0.415	1.5	0	0.00
Fluroxypyr	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Flutriafol	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Gamma - HCH	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Heptachlor	µg/l	8	<0.001	<0.001	<0.001	0.03	0	0.00
Heptachlor epoxide	µg/l	8	<0.002	<0.002	<0.002	0.03	0	0.00
Hexachlorobenzene	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Hexachlorobutadiene	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Ioxynil	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Iron	µg/l	52	<7.000	<13.481	82.000	200	0	0.00
Isodrin	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Isoproturon	µg/l	8	<0.002	<0.004	<0.004	0.1	0	0.00
Lead	µg/l	8	<0.500	<1.138	5.100	25	0	0.00
Linuron	µg/l	8	<0.003	<0.003	<0.005	0.1	0	0.00
Manganese	µg/l	52	<1.500	<1.546	2.600	50	0	0.00
MCPA	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
MCPB	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Mecoprop	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Mercury	µg/l	8	<0.012	<0.012	<0.012	1	0	0.00
Metamitron	µg/l	8	<0.002	<0.002	<0.003	0.1	0	0.00
Metazachlor	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Methabenzthiazuron	µg/l	8	<0.002	<0.002	<0.003	0.1	0	0.00
Methoxychlor	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Monolinuron	µg/l	8	<0.002	<0.002	<0.004	0.1	0	0.00
Nickel	µg/l	8	<0.900	<1.788	2.500	20	0	0.00
Nitrate	mg/l	8	10.200	14.775	17.900	50	0	0.00
Nitrite	mg/l	8	<0.002	<0.003	0.004	0.5	0	0.00
Nitrite/Nitrate Calculated	- ³	8	0.200	0.295	0.360	<1 ³	0	0.00
Odour	Dilution Number	26	0.000	0.000	0.000	3 at 25°C	0	0.00
PAH	µg/l	8	0.000	0.000	0.001	0.1	0	0.00
PCB - Arochlor 1254	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Pendimethalin	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
pH	pH value	52	7.260	7.719	8.240	Max 9.5, Min 6.5	0	0.00
Pirimicarb	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Prometryn	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Propachlor	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Propazine	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Propiconazole	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Propyzamide	µg/k	8	<0.002	<0.002	<0.002	0.1	0	0.00
Selenium	µg/l	8	0.420	0.670	1.200	10	0	0.00
Simazine	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Sodium	mg/l	8	21.000	27.250	35.000	200	0	0.00
T.D.E - Ortho Para	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
T.D.E - Para Para	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Taste	Dilution Number	26	0.000	0.000	0.000	3 at 25°C	0	0.00
Tecnazene	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Terbutryn	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Tetrachloroethene and Trichloroethene	µg/l	8	0.000	0.000	0.000	10	0	0.00
Tetrachloromethane	µg/l	8	<0.020	<0.030	<0.040	3	0	0.00
Total Pesticides	µg/l	8	0.000	0.000	0.001	0.5	0	0.00
Total Trihalomethanes	µg/l	8	22.200	34.138	50.600	100	0	0.00
Trans - Permethrin	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Triadimefon	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00

*PCV Prescribed concentration or value for mandatory parameters and specified limit or value for non-mandatory parameters (indicators and disinfectants).

*1 No numerical limit applies.

*2 Guidance levels only. Monitored for the calculation of Total Indicative Dose, which is the regulatory parameter. No samples exceeded the TID in 2007.

*3 The formula for the calculated Nitrate/Nitrite level is [Nitrate]/50 + [Nitrite]/3. This number has no units.

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 2 (continued) Typical Water Quality Characteristics

WATER SUPPLY ZONE ZDB13 ALVASTON								
REPORTING PERIOD: 01/01/2007 - 31/12/2007								
PARAMETER	ANALYSIS UNITS	NO. SAMPLES TAKEN	CONCENTRATION OR VALUE (ALL SAMPLES)			PCV*	SAMPLES CONTRAVENING PCV	
			MINIMUM	AVERAGE	MAXIMUM		NUMBER	PERCENT
Triallate	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Trichlorobenzene	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Triclopyr	µg/l	8	<0.003	<0.003	<0.003	0.1	0	0.00
Trietazine	µg/l	8	<0.001	<0.001	<0.001	0.1	0	0.00
Trifluralin	µg/l	8	<0.002	<0.002	<0.002	0.1	0	0.00
Turbidity	NTU	52	<0.050	<0.066	0.190	4	0	0.00
Free Chlorine	mg/l	180	0.030	0.231	0.550	- ¹	-	-
Total Chlorine	mg/l	180	0.080	0.297	0.610	- ¹	-	-
Ammonium	mg/l	52	<0.009	<0.012	0.021	0.5	0	0.00
Chloride	mg/l	8	17.600	24.788	32.500	250	0	0.00
Clostridium perfringens	No. / 100 ml	26	0.000	0.000	0.000	0	0	0.00
Coliform Bacteria	No. / 100 ml	180	0.000	0.000	0.000	0	0	0.00
Colony Counts after 48 hours at 37°C	No. / 100 ml	52	0.000	3.096	63.000	- ¹	-	-
Colony Count after 72 hours at 22°C	No. / 100 ml	52	0.000	7.654	142.000	- ¹	-	-
Conductivity	µS/cm at 20°C	26	251.000	421.385	542.000	2500	0	0.00
Gross Alpha Activity	Bq/l	8	0.063	0.087	0.125	0.1 ²	-	-
Gross Beta Activity	Bq/l	8	0.012	0.042	0.071	1 ²	-	-
Sulphate	mg/l	8	52.100	69.763	85.800	250	0	0.00
Total Organic Carbon	mg/l	8	0.810	1.041	1.380	- ¹	-	-

*PCV Prescribed concentration or value for mandatory parameters and specified limit or value for non-mandatory parameters (indicators and disinfectants).

*1 No numerical limit applies.

*2 Guidance levels only. Monitored for the calculation of Total Indicative Dose, which is the regulatory parameter. No samples exceeded the TID in 2007.

*3 The formula for the calculated Nitrate/Nitrite level is [Nitrate]/50 + [Nitrite]/3. This number has no units.

ACUTE TOXICITY TO RAINBOW TROUT**Appendix 3 Verification of Test Concentrations****1. METHOD OF ANALYSIS****1.1 Introduction**

The test material concentration in the test samples was determined by high performance liquid chromatography (HPLC) using an external standard. The test material gave a chromatographic profile consisting of a number of peaks. The results have been calculated using the main peak associated with the test material.

The method was developed by the Department of Analytical Services, Harlan Laboratories Ltd, Shardlow, UK.

1.2 Sample Preparation

A C8 (EC) solid phase extraction (SPE) cartridge (500mg/ 3ml) was sequentially pre-conditioned with methanol and water*. A volume of test sample was eluted through the cartridge and the cartridge dried. The test material was eluted from the cartridge with methanol and made to volume to give a final theoretical concentration of approximately 0.40 mg/l.

1.3 Standards

Standard solutions of test material were prepared initially in tetrahydrofuran at a nominal concentration of 1000 mg/l. The standards were then diluted using methanol to give a final theoretical concentration of 0.5 mg/l in methanol containing 10% tetrahydrofuran.

* Prepared by ELGA Purelab Option R-15 water purification

ACUTE TOXICITY TO RAINBOW TROUT**Appendix 3 (continued) Verification of Test Concentrations****1.4 Procedure**

The standards and samples were analysed by HPLC using the following conditions:

HPLC System	:	Agilent Technologies 1200, incorporating autosampler and workstation
Column	:	Symmetry, C18, 3.5 μ , (50 x 3.0 mm id)
Column temperature	:	40°C
Mobile phase	:	methanol
Flow rate	:	0.8 ml/min
UV/Vis detector wavelength	:	210 nm
Injection volume	:	50 μ l
Retention time	:	approximately 1.4 minutes

2. PRE-STUDY MEDIA PREPARATION TRIALS**2.1 Saturated Solution Preparation**

An amount of test material (550 mg) was dispersed, in duplicate, in 11 litres of reconstituted water. These were stirred using a propeller stirrer at approximately 1500 rpm at approximately 21°C for periods of 24 and 48 hours.

Samples were taken for analysis following removal of any undissolved test material by centrifugation at 10000 or 40000 *g* for 30 minutes or following filtration through 0.2 μ m Sartorius Sartopore filters with the first 500 or 1 litre being discarded.

Stirring Period and Treatment	Concentration Found (mg/l)
24 Hours Control	<LOQ
24 Hours Centrifuged 10000 <i>g</i>	0.00410
24 Hours Centrifuged 40000 <i>g</i>	0.00261
24 Hours Filtered 500 ml discarded	<LOQ
24 Hours Filtered 1 litre discarded	<LOQ

LOQ = Limit of quantitation

ACUTE TOXICITY TO RAINBOW TROUT**Appendix 3 (continued) Verification of Test Concentrations**

Stirring Period and Treatment	Concentration Found (mg/l)
48 Hours Control	<LOQ
48 Hours Centrifuged 10000 g	0.00539
48 Hours Centrifuged 40000 g	0.00624
48 Hours Filtered 500 ml discarded	<LOQ
48 Hours Filtered 1000 ml discarded	<LOQ

The above results have been corrected for a recovery rate of 79%.

3. VALIDATION**3.1 Linearity**

A range of standard solutions covering 0.025 to 0.74 mg/l (exceeding the range of the working sample concentrations) was analysed.

Linearity was confirmed ($R^2 = 0.9981$) in the range 0 to 0.74 mg/l.

The results are presented graphically on page 27.

3.2 Recoveries

Preliminary test samples, accurately fortified at a known concentration of test material, were prepared and analysed.

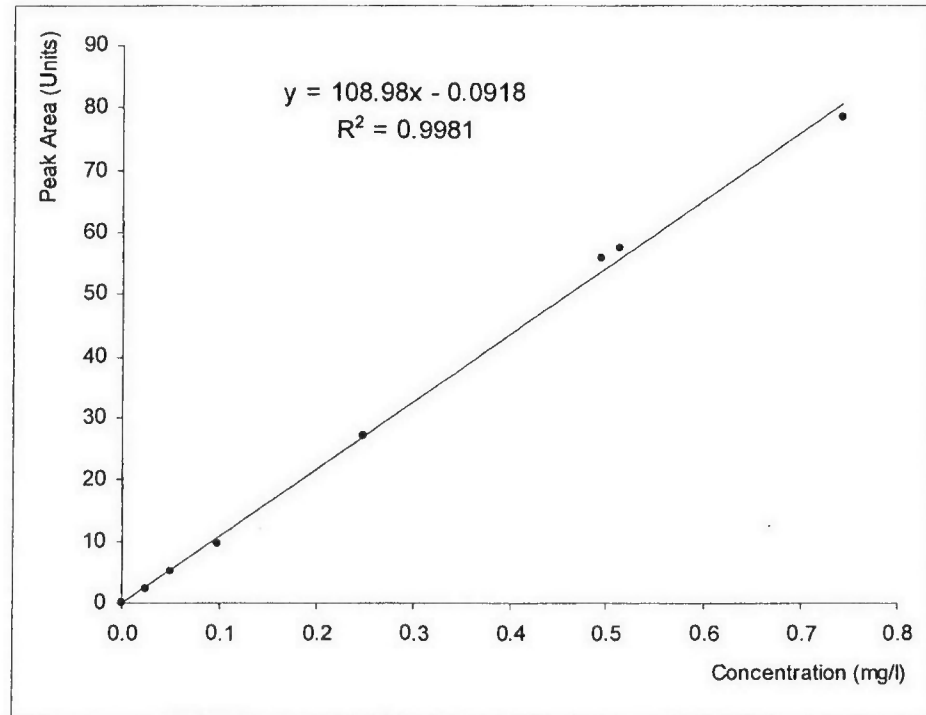
The recovery samples were prepared by addition of a standard solution of test material to a sample of test medium. A standard solution was accurately prepared by dissolving the test material initially in tetrahydrofuran then with methanol. An accurate volume of the standard solution was added to a known volume of test medium to achieve the required concentration of test material.

LOQ = Limit of quantitation

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

Linearity of Detector Response



ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

Fortification (mg/l)	Recoveries		
	(mg/l)	(%)	Mean %
0.00408	0.00297	73	78.137
0.00408	0.00315	77	
0.00408	0.00331	81	
0.00408	0.00328	81	
0.00408	0.00323	79	

The recovery results were below the acceptance limits of 80 - 120% therefore all test sample results were corrected for an overall mean recovery rate of 78.137%

The method has been considered to be sufficiently accurate for the purposes of this test.

The limit of quantitation has been assessed down 0.00025 mg/l.

4. STABILITY

Preliminary test samples were prepared, analysed initially and then after storage in sealed glass vessels at ambient temperature in light and dark conditions for approximately 24 hours (equivalent to the period of media renewal). In addition a test sample was tested for stability without prior mixing (sonication) of the test sample bottle to assess for losses due to adsorption and/or insolubility.

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

Nominal concentration (mg/l)	0.00041
Concentration found initially (mg/l)	0.00319
Concentration found after storage in light conditions (mg/l)	0.00274
Expressed as a percent of the initial concentration	86
Concentration found after storage in dark conditions (mg/l)	0.00183
Expressed as a percent of the initial concentration	57
Concentration found after storage in dark conditions (mg/l) – unsonicated sample	0.00324
Expressed as a percent of the initial concentration	101

The test samples have been shown to be stable in the test medium in light and dark conditions.

The test sample result for the dark stability is believed to be erroneous as all other stability results show the test material to be stable.

The unsonicated stability vessel showed no evidence of insolubility or adherence to glass.

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

5. RESULTS

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
0 hours Fresh Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00559	140
	0.0040 U R ₂	0.00581	145
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00326	82
	0.0040 C R ₂	0.00362	91
24 hours Old Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00503	126
	0.0040 U R ₂	0.00517	129
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00346	86
	0.0040 C R ₂	0.00319	80
24 hours Fresh Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00594	148
	0.0040 U R ₂	0.00608	152
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00424	106
	0.0040 C R ₂	0.00471	118
48 hours Old Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00461	115
	0.0040 U R ₂	0.00434	108
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00273	68
	0.0040 C R ₂	0.00229	57

* Results corrected for an overall mean recovery rate of 78.137% taken from the validation.

LOQ = Limit of quantitation

U = untreated

C = centrifuged at 10000g for 30 minutes in polycarbonate tubes at 21°C.

R₁ - R₂ = Replicates 1 to 2

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
48 hours Fresh Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00605	151
	0.0040 U R ₂	0.00599	150
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00463	116
	0.0040 C R ₂	0.00438	109
72 hours Old Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00447	112
	0.0040 U R ₂	0.00466	116
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00256	64
	0.0040 C R ₂	0.00292	73
72 hours Fresh Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00561	140
	0.0040 U R ₂	0.00545	136
	Solvent control C	0.000390 / <LOQ**	- / -*
	0.0040 C R ₁	0.00424	106
	0.0040 C R ₂	0.00390	98
96 hours Old Media	Solvent control U	<LOQ	-
	0.0040 U R ₁	0.00424	106
	0.0040 U R ₂	0.00509	127
	Solvent control C	<LOQ	-
	0.0040 C R ₁	0.00257	64
	0.0040 C R ₂	0.00319	80

* Results corrected for an overall mean recovery rate of 78.137% taken from the validation.

LOQ = Limit of quantitation

U = untreated

C = centrifuged at 10000g for 30 minutes in polycarbonate tubes at 21°C.

** Duplicate sample, stored frozen prior to analysis

R₁ - R₂ = Replicates 1 to 2

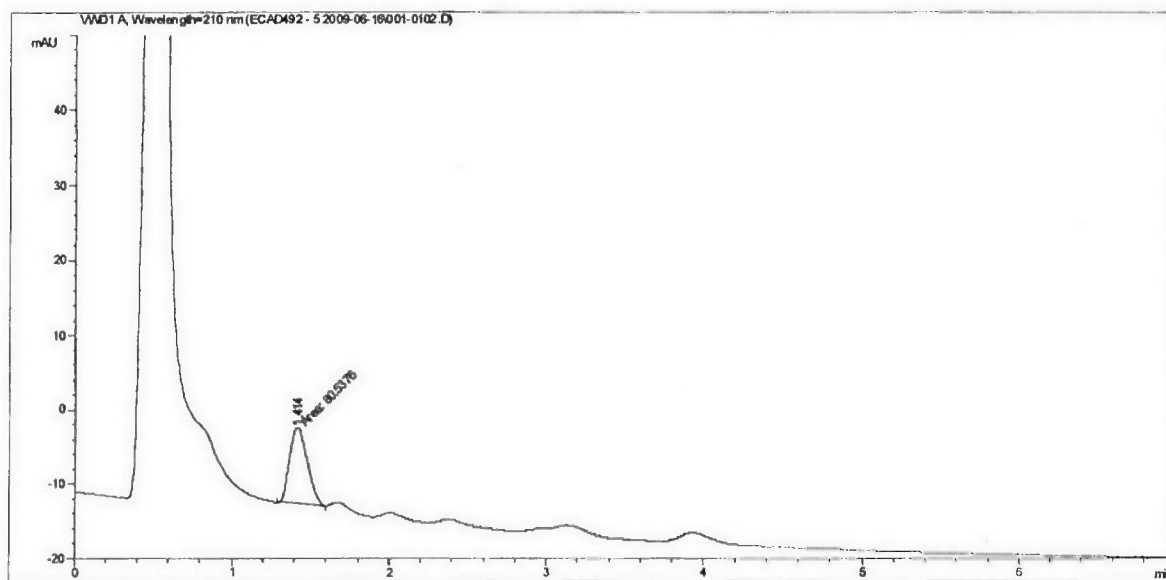
ACUTE TOXICITY TO RAINBOW TROUT**Appendix 3 (continued) Verification of Test Concentrations****6. DISCUSSION**

The detection system was found to have acceptable linearity. The analytical procedure had low recoveries of test material in test medium which was overcome by correction of an overall recovery rate of 78.137%. A method of analysis was validated and proven to be suitable for use.

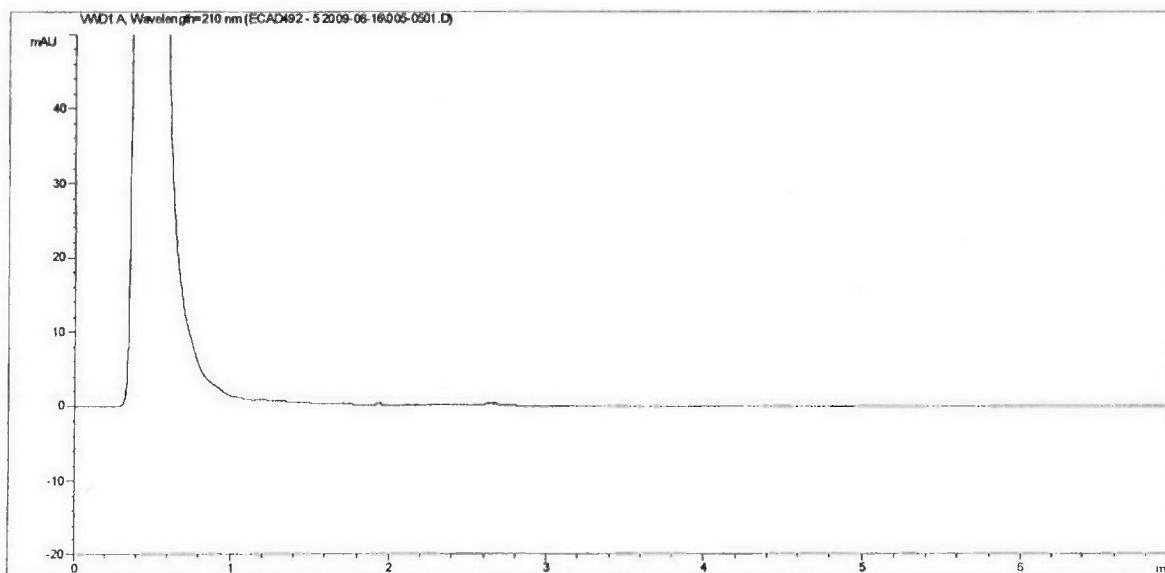
ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

7. TYPICAL CHROMATOGRAPHY



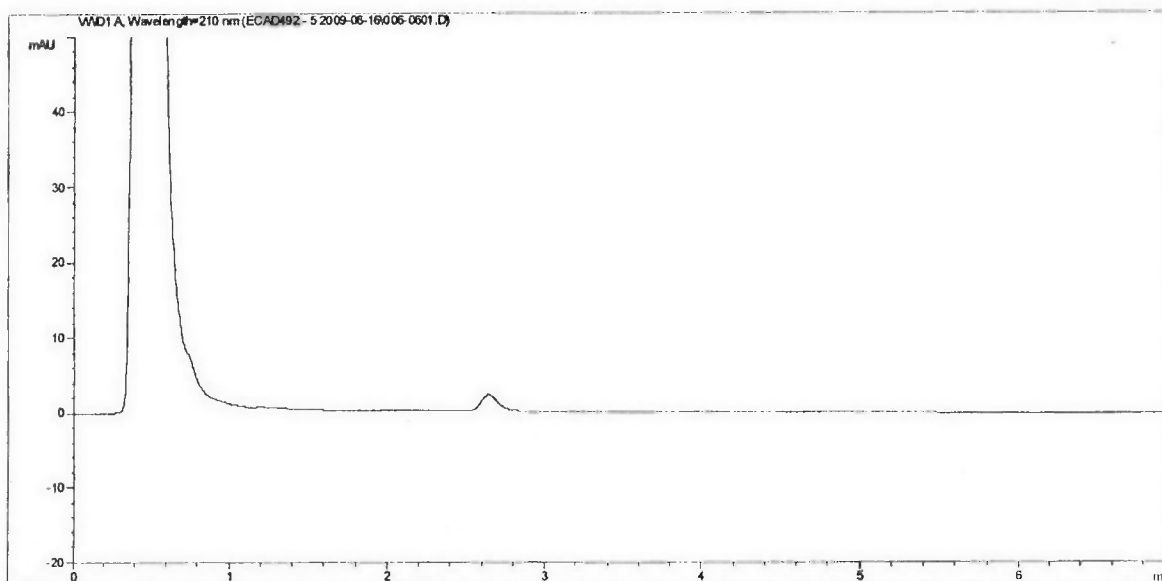
Standard 0.50 mg/l



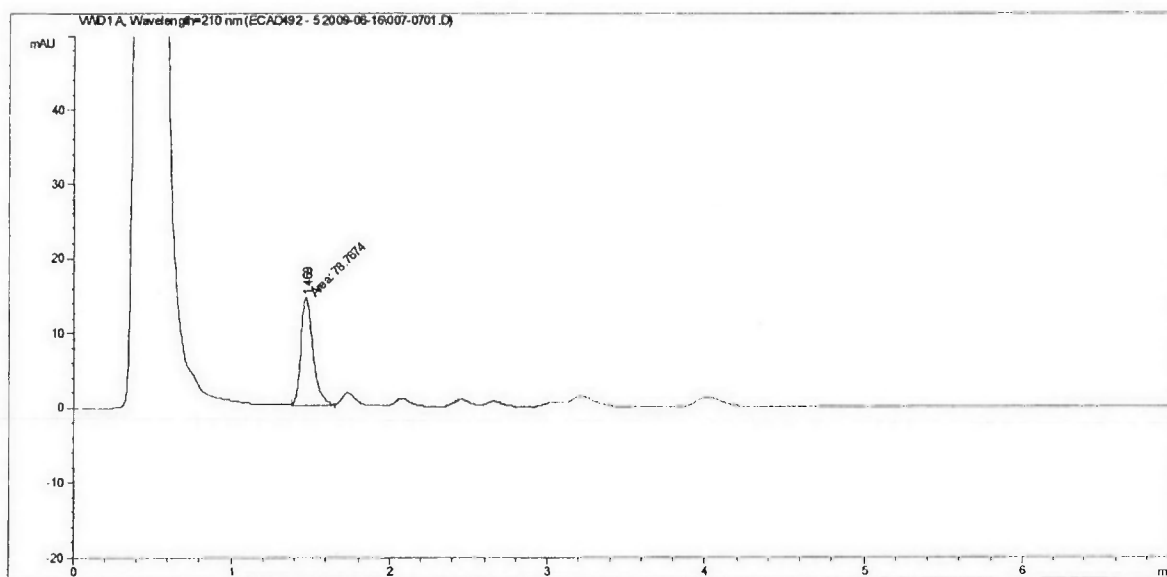
Control Sample Untreated

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

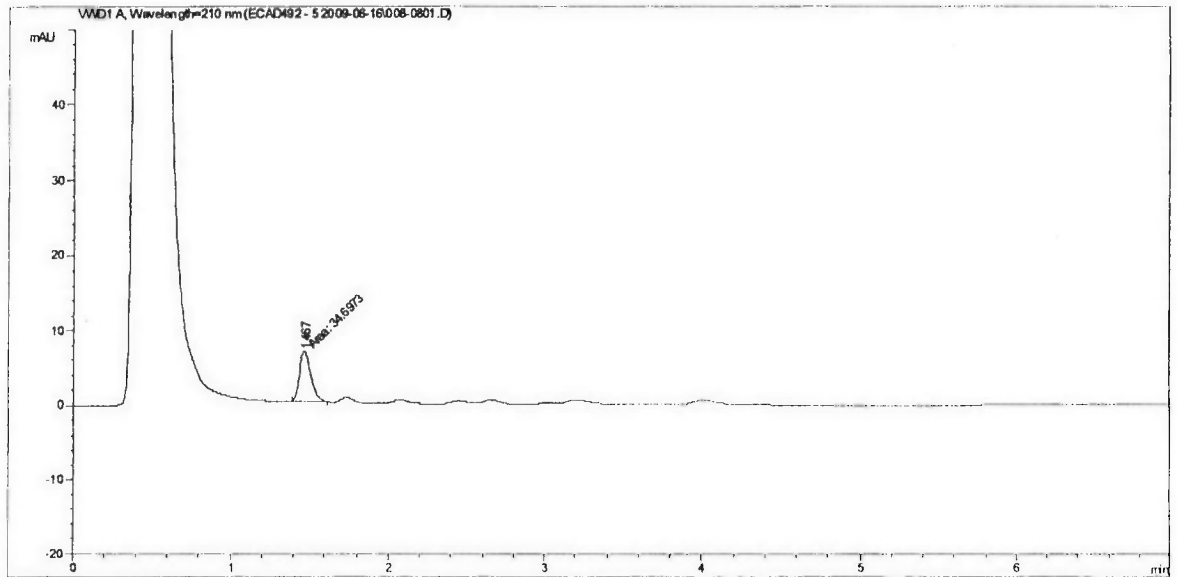
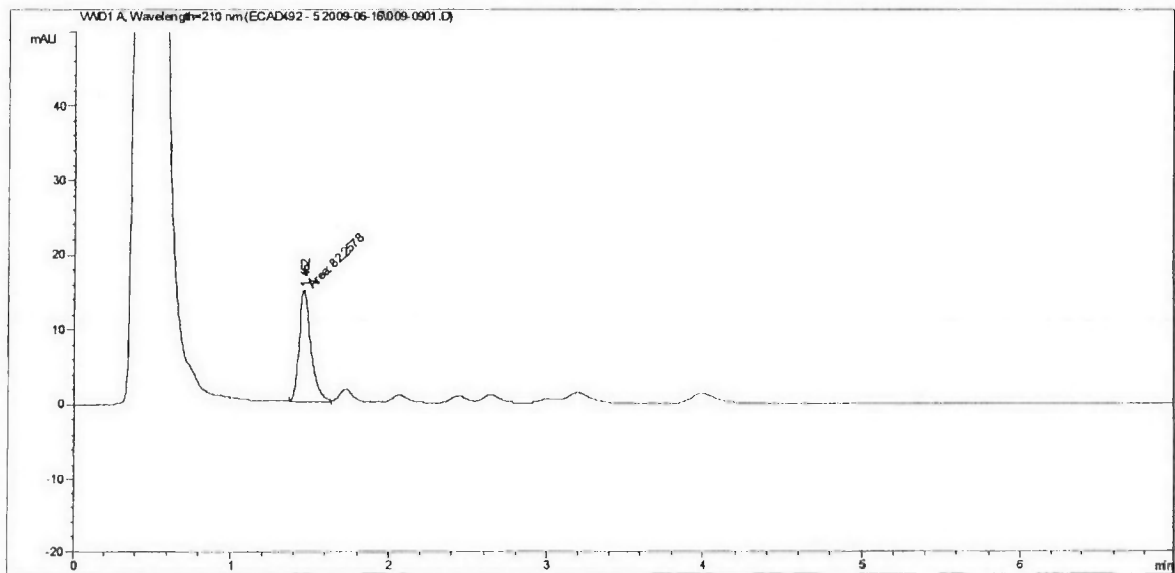


Control Sample Centrifuged

Test Sample 0.0040 mg/l R₁ Untreated

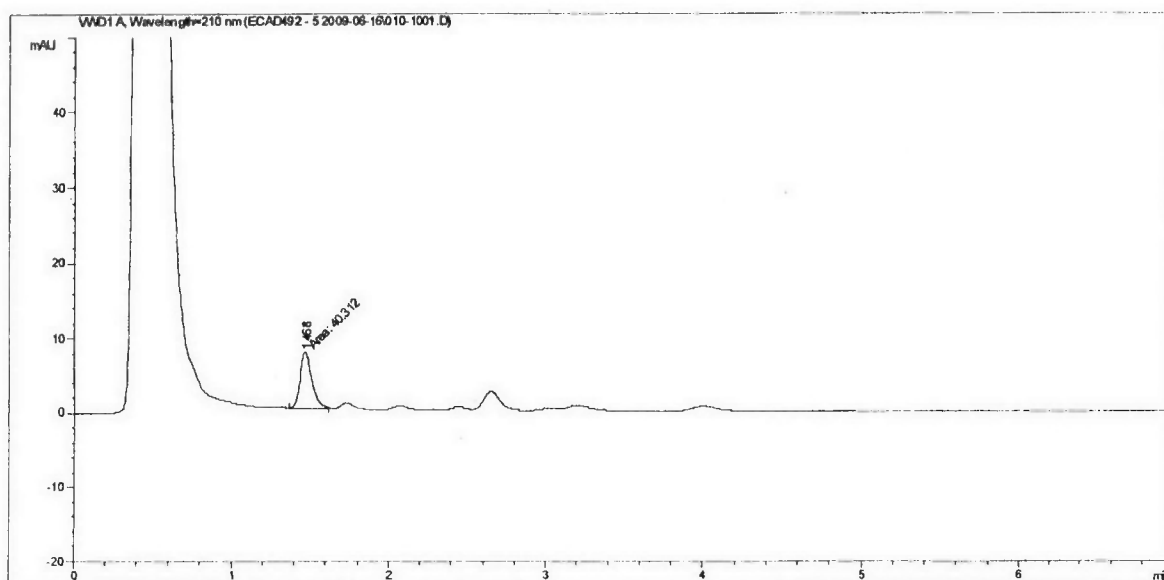
ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations

Test Sample 0.0040 mg/l R₁ CentrifugedTest Sample 0.0040 mg/l R₂ Untreated

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 (continued) Verification of Test Concentrations



Test Sample 0.0040 mg/l R₂ Centrifuged

ACUTE TOXICITY TO RAINBOW TROUT

Appendix 4 Physico-Chemical Measurements

Time-weighted mean Measured Test Concentration (mg/l)	Time (Hours)											
	0 Hours (Fresh Media)				24 Hours (Old Media)				24 Hours (Fresh Media)			
	pH	mg O ₂ /l	%ASV*	T°C	pH	mg O ₂ /l	%ASV*	T°C	pH	mg O ₂ /l	%ASV*	T°C
Control	7.6	9.9	96	14	8.0	10.1	98	14	7.6	9.9	98	15
Solvent Control	7.7	9.9	96	14	8.0	10.0	97	14	7.6	9.9	98	15
0.0034 R ₁	7.7	9.9	96	14	8.0	10.0	97	14	7.6	9.9	98	15
0.0034 R ₂	7.6	10.0	97	14	8.0	10.0	97	14	7.6	9.9	98	15

Time-weighted mean Measured Test Concentration (mg/l)	Time (Hours)											
	48 Hours (Old Media)				48 Hours (Fresh Media)				72 Hours (Old Media)			
	pH	mg O ₂ /l	%ASV*	T°C	pH	mg O ₂ /l	%ASV*	T°C	pH	mg O ₂ /l	%ASV*	T°C
Control	8.0	10.1	100	15	7.7	9.9	98	15	7.8	10.0	99	15
Solvent Control	8.0	10.0	99	15	7.6	9.9	98	15	7.9	10.1	100	15
0.0034 R ₁	8.0	10.1	100	15	7.6	9.9	98	14	7.9	10.1	100	15
0.0034 R ₂	8.0	10.0	99	15	7.6	9.9	98	15	7.9	10.1	100	15

Time-weighted mean Measured Test Concentration (mg/l)	Time (Hours)							
	72 Hours (Fresh Media)				96 Hours (Old Media)			
	pH	mg O ₂ /l	%ASV*	T°C	pH	mg O ₂ /l	%ASV*	T°C
Control	7.7	10.0	99	15	7.9	10.0	99	15
Solvent Control	7.7	10.0	99	15	7.9	10.1	100	15
0.0034 R ₁	7.7	9.9	98	15	8.0	10.0	99	15
0.0034 R ₂	7.7	9.9	98	15	8.0	10.0	99	15

*ASV = Dissolved oxygen concentration expressed as a percentage of Air Saturation Value
R₁ and R₂ = Replicates 1 and 2

**Appendix 5 Statement of GLP Compliance in Accordance with Directive
2004/9/EC**



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

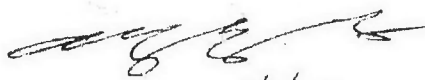
Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


4/3/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



ACUTE TOXICITY TO *DAPHNIA MAGNA*

PROJECT NUMBER: 2737/0002

AUTHOR: S L Priestly

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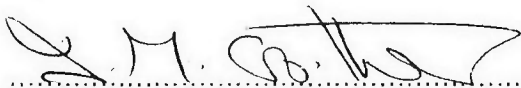
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

13 November 2008	Standard Test Method Compliance Audit
11, 18 May 2009	Test Material Preparation
19 May 2009	Test System Preparation
19 May 2009	Exposure
22 May 2009	Assessment of Response
13, 15 May 2009	Chemical Analysis
§ 18 June 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§ Evaluation specific to this study	



For the Quality Assurance Unit*

DATE: 26 JUN 2009

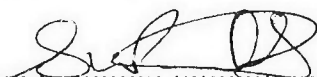
***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.



Date: 25 JUN 2009

S L Priestly BSc
Study Director

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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ACUTE TOXICITY TO *DAPHNIA MAGNA*

SUMMARY

Introduction. A study was performed to assess the acute toxicity of the test material to *Daphnia magna*. The method followed that described in the OECD Guidelines for Testing of Chemicals (April 2004) No 202, "*Daphnia* sp, Acute Immobilisation Test" referenced as Method C.2 of Commission Regulation (EC) No. 440/2008.

Methods. Information provided by the Sponsor indicated that the test material was insoluble in water. Pre-study solubility work conducted indicated that it was not possible to obtain a testable solution of the test material using traditional methods of preparation e.g. ultrasonication and high shear mixing.

A pre-study media preparation trial indicated that a dissolved test material concentration of approximately 0.0041 mg/l was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this material under test conditions.

Chemical analysis of the test preparations at 0 hours showed measured concentrations to be lower than 0.0041 mg/l, this was considered to be due to slight differences in media and/or sampling techniques used to remove the supernatant after centrifugation between the media preparation trial and the definitive test.

Based on the results of a preliminary range-finding test, twenty daphnids (4 replicates of 5 animals) were exposed to an aqueous solution of the test material at a 0-Hour measured concentration of 0.0016 mg/l for 48 hours at a temperature of 20 to 22°C under static test conditions. The test material solutions were prepared by stirring an excess (50 mg/l) of test material in reconstituted water using a propeller stirrer at approximately 1500 rpm at a temperature of approximately 20°C for 24 hours. After the stirring period any undissolved test material was removed by centrifugation at 10000 g for 30 minutes to produce a saturated solution of the test material with a 0-Hour measured test concentration of 0.0016 mg/l. Immobilisation and any adverse reactions to exposure were recorded after 24 and 48 hours.

A positive control conducted approximately every six months used potassium dichromate as the reference material. *Daphnia magna* was exposed to an aqueous solution of the reference material at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l for 48 hours at a temperature of approximately 20°C under static test conditions. Immobilisation and any adverse reactions to exposure were recorded after 3, 24 and 48 hours.

Results. The 48-Hour EC_{50} based on the 0-Hour measured test concentrations was greater than 0.0016 mg/l and correspondingly the No Observed Effect Concentration was 0.0016 mg/l.

Analysis of the test preparations at 0 hours showed measured test concentrations to range from 0.00161 mg/l to 0.00167 mg/l.

Analysis of the old or expired test preparations at 48 hours showed a slight decline in measured test concentrations of 0.000514 mg/l to 0.00106 mg/l.

A Study to Determine the Partition Coefficient of the test material (Harlan Laboratories Ltd Project Number: 2737/0004) gave $\log_{10} P_{ow}$ value of greater than 6.5. Therefore it was considered that the decline in measured concentration over the 48-Hour test period was due to possible bioaccumulation.

Given this decline in measured test concentrations it was considered justifiable to base the results on the geometric mean measured test concentrations test media in order to give a "worst case" analysis of the data.

The 48-Hour EC_{50} based on the geometric mean measured test concentrations was greater than 0.0011 mg/l and correspondingly the No Observed Effect Concentration was 0.0011 mg/l.

This study showed that there were no toxic effects at saturation.

The 48-Hour EC_{50} for the reference material to *Daphnia magna* based on nominal concentrations was 0.71 mg/l with 95% confidence limits of 0.61 – 0.81 mg/l. The No Observed Effect Concentration was 0.32 mg/l.

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ACUTE TOXICITY TO *DAPHNIA MAGNA*

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of the test material to *Daphnia magna*. The method followed the recommendations of the OECD Guidelines for Testing of Chemicals (April 2004) No 202 "*Daphnia* sp, Acute Immobilisation Test" referenced as Method C.2 of Commission Regulation (EC) No. 440/2008.

Daphnia magna is a freshwater invertebrate representative of a wide variety of natural habitats, and can therefore be considered as an important non-target organism in freshwater ecosystems.


The study was conducted between 27 April 2009 and 22 May 2009.

The positive control (Harlan Laboratories Ltd Project No: 0039/1069) was conducted between 9 December 2008 and 11 December 2008.

In view of the difficulties associated with the evaluation of aquatic toxicity of poorly water soluble test materials, a modification of the standard method for the preparation of aqueous media was performed. An approach endorsed by several important regulatory authorities in the EU and elsewhere (ECETOC 1996 and OECD 2000), is to expose organisms to a saturated solution of the test material in cases where the test material is of high purity and is poorly soluble in water and in the permitted auxiliary solvents and surfactants. Using this approach, a saturated solution was prepared by stirring an excess (50 mg/l) of test material in reconstituted water for a period of 24 hours prior to removing any undissolved test material present by centrifugation at 10000 g for 30 minutes to give a saturated solution of the test material.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification : 

Description : white powder

Batch number : 20090105

Date received : 23 January 2009

Storage conditions : room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor. A Certificate of Analysis is included as Appendix 1.

3. METHODS

3.1 Test Species

The test was carried out using 1st instar *Daphnia magna* derived from in-house laboratory cultures.

Adult *Daphnia* were maintained in polypropylene vessels containing approximately 2 litres of reconstituted water in a temperature controlled room at approximately 20°C. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle with 20 minute dawn and dusk transition periods. Each culture was fed daily with a suspension of algae (*Chlorella* sp.). Culture conditions ensured that reproduction was by parthenogenesis. Gravid adults were isolated the day before initiation of the test, such that the young daphnids produced overnight were less than 24 hours old. These young were removed from the cultures and used for testing. The diet and diluent water are considered not to contain any contaminant that would affect the integrity or outcome of the study.

3.2 Test Water

The reconstituted water used for both the range-finding and definitive tests was the same as that used to maintain the stock animals.

The reconstituted water is defined in Appendix 2.

3.3 Procedure

3.3.1 Pre-study Media Preparation Trial

Information provided by the Sponsor indicated that the test material was insoluble in water. Pre-study solubility work conducted indicated that it was not possible to obtain a testable solution of the test material using traditional methods of preparation e.g. ultrasonication and high shear mixing.

Preliminary solubility work conducted indicated that the test material was practically insoluble in water using traditional methods of preparation e.g. ultrasonication and high shear mixing.

Based on this information the test material was categorised as being a 'difficult substance' as defined by the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 2000). Therefore a media preparation trial was conducted in order to determine the solubility of the test material under test conditions.

An amount of test material (550 mg) was dispersed, in duplicate, in 11 litres of reconstituted water with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 21°C for 24 or 48 hours. After the stirring periods samples were taken for chemical analysis after the following pre-treatments:

- Centrifugation at 10000 g for 30 minutes
- Centrifugation at 40000 g for 30 minutes
- Filtration through a 0.2 µm Sartorius Sartopore filter (approximate 500 ml discarded in order to pre-condition the filter)
- Filtration through a 0.2 µm Sartorius Sartopore filter (approximate 1 litre discarded in order to pre-condition the filter)

3.3.2 Range-finding test

The test concentration to be used in the definitive test was determined by a preliminary range-finding test.

In the range-finding test *Daphnia magna* were exposed to a series of nominal test concentrations of 0.000041, 0.00041 and 0.0041 mg/l*. The test material was prepared from a saturated solution.

An amount of test material (550 mg) was dispersed in 11 litres of reconstituted water with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 20°C for 24 hours. After 24 hours the stirring was stopped and any undissolved test material was removed by centrifugation at 10000 g for 30 minutes to give a saturated solution with a nominal test concentration of 0.0041 mg/l*. Serial dilutions were prepared in reconstituted water to give the remainder of the test series of 0.00041 and 0.000041 mg/l.

Each stock solution and prepared concentration was inverted several times to ensure adequate mixing and homogeneity.

In the range-finding test 10 daphnids were placed in each test and control vessel and maintained in a temperature controlled room at 21 to 22°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 48 hours with 20 minute dawn and dusk transition periods. The temperature at 48 hours was observed to be slightly in excess of the 20°C \pm 1°C range given in the Protocol. This deviation was considered not to have any impact on the outcome or validity of the test as no adverse effects of exposure were observed throughout the test. Each 250 ml test and control vessel contained 200 ml of test media and was covered to reduce evaporation. After 24 and 48 hours the number of immobilised *Daphnia magna* were recorded.

The control group was maintained under identical conditions but not exposed to the test material.

3.3.3 Definitive test

Based on the results of the pre-study media preparation trials and range-finding test a "Limit test" was conducted at a nominal concentration of 0.0041 mg/l to confirm that at

* Concentration based on the results of the pre-study media preparation trial.

the highest attainable test concentration of 0.0041 mg/l, no significant immobilisation or adverse reactions to exposure were observed.

Chemical analysis of the test preparations at 0 hours showed measured concentrations to be lower than 0.0041 mg/l, this was considered to be due to slight differences in media and/or sampling techniques used to remove the supernatant after centrifugation between the media preparation trial and the definitive test. It was therefore considered appropriate to express the concentrations in the definitive test in terms of the 0-Hour measured concentration.

3.3.3.1 *Experimental preparation*

For the purpose of the definitive test the test material was prepared as a saturated solution in reconstituted water.

An amount of test material (550 mg) was dispersed in 11 litres of reconstituted water with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 20°C for 24 hours. After 24 hours the stirring was stopped and any undissolved test material was removed by centrifugation at 10000 g for 30 minutes to give a saturated solution with a 0-Hour measured test concentration of 0.0016 mg/l.

The concentration and stability of the test material in the test preparations were verified by chemical analysis at 0 and 48 hours (see Appendix 3).

3.3.3.2 *Exposure conditions*

In the definitive test 250 ml glass jars containing approximately 250 ml of test preparation were used. At the start of the study 5 daphnids were placed in each test and control vessel at random, in the test preparations. Four replicate test and control vessels were prepared. The test vessels were then covered to reduce evaporation and maintained in a temperature controlled room at 21 to 22°C with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods. The daphnids were not individually identified, received no food during exposure and the test vessels were not aerated.

The control group was maintained under identical conditions but not exposed to the test material.

The test preparations were not renewed during the exposure period. Any immobilisation or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure. The criterion of effect used was that *Daphnia* were considered to be immobilised if they were unable to swim for approximately 15 seconds after gentle agitation.

3.3.3.3 Physico-chemical measurements

Water temperature was recorded daily throughout the test. Dissolved oxygen concentrations and pH were recorded at the start and termination of the test. The pH and dissolved oxygen concentration were measured using a WTW pH/Oxi 340I pH and dissolved oxygen meter and the temperature was measured using a Hanna Instruments HI 93510 digital thermometer.

3.3.3.4 Verification of test concentrations

Water samples were taken from the control (replicates $R_1 - R_2$ pooled) and the 0.0016 mg/l test group (replicates $R_1 - R_2$ and $R_3 - R_4$ pooled) at 0 and 48 hours for quantitative analysis.

Duplicate samples were taken and stored at approximately -20°C for further analysis if necessary.

The method of analysis, stability, recovery and test preparation analyses are described in Appendix 3.

3.3.3.5 Evaluation of data

An estimate of the EC_{50} values was given by inspection of the immobilisation data.

The geometric mean measured test concentrations of replicates R_1 and R_2 pooled and replicates R_3 and R_4 pooled were calculated as follows:

$$GM = \sqrt{C_0 C_1}$$

where:

GM = geometric mean measured test concentration (mg/l)
C₀ = measured concentration at the start of the test (mg/l)
C₁ = measured concentration at the end of the test (mg/l)

3.3.4 Positive Control

A positive control (Harlan Laboratories Ltd Project No: 0039/1069) conducted approximately every six months used potassium dichromate as the reference material at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l.

An amount of reference material (100 mg) was dissolved in reconstituted water and the volume adjusted to 1 litre to give a 100 mg/l stock solution. An aliquot (50 ml) of this stock solution was diluted in reconstituted water and the volume adjusted to 500 ml to give a 10 mg/l stock solution. Aliquots (16, 28, 50, 90 and 160 ml) of the 10 mg/l stock solution were each separately dispersed in a final volume of 500 ml of reconstituted water to give the test series of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/l respectively.

Each stock solution and prepared concentration was inverted several times to ensure adequate mixing and homogeneity.

Exposure conditions for the positive control were similar to those used in the definitive test.

The temperature was maintained at approximately 20°C.

3.3.5 Evaluation of data for the positive control

An estimate of the EC₅₀ value at 3 hours was given by inspection of the immobilisation data.

The EC₅₀ values and associated confidence limits at 24 and 48 hours and the slope of the response curves and standard errors were calculated by the maximum-likelihood

probit method (Finney 1971) using the ToxCalc computer software package (ToxCalc 1999).

Probit analysis is used where two or more partial responses to exposure are shown.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Pre-Study Media Preparation Trial

The results obtained from the pre-study media preparation trial conducted (see Appendix 3) indicated that the test material was adsorbing to the filter matrices. There was no significant increase in the dissolved test material concentration obtained when the preparation period of the saturated solution was extended to 48 hours.

Based on this information the test material was prepared using a saturated solution method of preparation at an initial loading rate of 50 mg/l, stirred for a period of 24 hours prior to removal of any undissolved test material by centrifugation at 10000 g for 30 minutes to give a nominal test concentration of approximately 0.0041 mg/l.

5.2 Range-finding Test

Cumulative immobilisation data from the exposure of *Daphnia magna* to the test material during the range-finding test are given in Table 1.

No immobilisation was observed at the nominal test concentration of 0.00041 mg/l with no significant immobilisation being observed at the test concentrations of 0.000041 and 0.0041 mg/l.

Based on this information, a single nominal test concentration of four replicates, of 0.0041 mg/l was selected for the definitive test. This experimental design conforms to a "Limit test" to confirm that at the highest attainable test concentration of 0.0041 mg/l, no significant immobilisation or adverse reactions to exposure were observed.

Chemical analysis of the test preparations at 0 hours in the definitive test showed measured concentrations to be lower than 0.0041 mg/l, this was considered to be due to slight differences in media and/or sampling techniques used to remove the supernatant after centrifugation between the media preparation trial and the definitive test. It was therefore considered appropriate to express the concentrations in the definitive test in terms of the 0-Hour measured concentration.

5.3 Definitive Test

5.3.1 Immobilisation data

Cumulative immobilisation data from the exposure of *Daphnia magna* to the test material during the definitive test are given in Table 2.

There was no significant immobilisation in 20 daphnids exposed to a 0-Hour measured test concentration of 0.0016 mg/l for a period of 48 hours. Inspection of the immobilisation data gave the following results:

Time (h)	EC ₅₀ (mg/l)	95% Confidence limits (mg/l)
24	>0.0016	-
48	>0.0016	-

The No Observed Effect Concentration after 24 and 48 hours exposure was 0.0016 mg/l. The No Observed Effect Concentration is based upon zero immobilisation at this concentration.

The test concentration of 0.0016 mg/l was the highest attainable test concentration that could be prepared due to the limited solubility of the test material in water.

5.3.2 Observations on test material solubility

The test preparations were observed to be clear, colourless solutions throughout the duration of the test.

5.3.3 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix 4. Temperature was maintained at 20 to 22°C throughout the test, while there were no treatment related differences for oxygen concentration or pH.

The temperature throughout the test was observed to be slightly in excess of the 20°C ± 1°C range given in the Protocol. This deviation was considered not to have any impact on the outcome or validity of the test as no adverse effects of exposure were observed throughout the test.

5.3.4 Verification of test concentrations

Analysis of the test preparations at 0 hours (see Appendix 3) showed measured test concentrations to range from 0.00161 mg/l to 0.00167 mg/l.

Analysis of the old or expired test preparations at 48 hours (see Appendix 3) showed a slight decline in measured test concentrations of 0.000514 mg/l to 0.00106 mg/l.

A Study to Determine the Partition Coefficient of the test material (Harlan Laboratories Ltd Project Number: 2737/0004) gave $\log_{10} P_{ow}$ value of greater than 6.5. Therefore it was considered that the decline in measured concentration over the 48-Hour test period was due to possible bioaccumulation.

Current regulatory advice is that in cases where a decline in measured concentrations is observed, geometric mean measured concentrations should be used for calculating EC_{50} values. It was therefore considered justifiable to base the results on the geometric mean measured test concentrations in order to give a "worst case" analysis of the data. The geometric mean measured test concentrations were determined to be:

0-Hour Measured Test Concentration (mg/l)	Geometric Mean Measured Test Concentration (mg/l)	% of the 0-Hour Measured Test Concentration
0.0016	0.0011	69

The following results were determined from the data based on the geometric mean measured test concentrations:

Time (h)	EC_{50} (mg/l)	95% Confidence limits (mg/l)
24	>0.0011	-
48	>0.0011	-

The use of geometric mean measured test concentrations was considered not to have affected the results of the test.

5.4 Positive Control

Cumulative immobilisation data from the exposure of *Daphnia magna* to the reference material (Harlan Laboratories Ltd Project No: 0039/1069) during the positive control are

given in Table 3. The relationship between percentage immobilisation and concentration at 24 and 48 hours is given in Figures 1 and 2.

Inspection of the immobilisation data at 3 hours and analysis of the immobilisation data by the probit method (Finney 1971) at 48 hours based on the nominal test concentrations gave the following results:

Time (h)	EC ₅₀ (mg/l)	95% Confidence limits (mg/l)
3	> 3.2	-
24	0.82	0.71 - 0.94
48	0.71	0.61 - 0.81

The No Observed Effect Concentration after 24 and 48 hours was 0.32 mg/l. The No Observed Effect Concentration is based upon zero immobilisation at this concentration.

The slopes and their standard errors of the response curves at 24 and 48 hours were 8.1 (SE = 1.7) and 8.6 (SE = 1.8) respectively.

The results from the positive control with potassium dichromate were within the normal range for this reference material. The mean 48-Hour EC₅₀ value calculated from all positive controls was 0.78 mg/l (sd = 0.21).

6. CONCLUSION

The acute toxicity of the test material to the freshwater invertebrate *Daphnia magna* has been investigated and based on the 0-Hour measured test concentration gave a 48-Hour EC₅₀ of greater than 0.0016 mg/l. Correspondingly the No Observed Effect Concentration was 0.0016 mg/l.

The 48-Hour EC₅₀ based on the geometric mean measured test concentrations was greater than 0.0011 mg/l and correspondingly the No Observed Effect Concentration was 0.0011 mg/l.

This study showed that there were no toxic effects at saturation.

7. REFERENCES

Environment Directorate, Organisation for Economic Co-operation and Development (OECD) (2000) Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures.

European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Monograph No. 26 (1996) Aquatic Toxicity Testing of Sparingly Soluble, Volatile and Unstable Substances.

Finney, D J (1971) Statistical Method in Biological Assay. London : Griffin and Company Ltd.

ToxCalc Version 5.0.23C (1999), Tidepool Scientific Software, McKinleyville, CA 95519, USA.

ACUTE TOXICITY TO *DAPHNIA MAGNA***Table 1** Cumulative Immobilisation Data in the Range-finding Test

Nominal Concentration (mg/l)	Cumulative Immobilised <i>Daphnia</i> (Initial Population: 10 Per Replicate)	
	24 Hours	48 Hours
Control	0	0
0.000041	1	1
0.00041	0	0
0.0041	1	1

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ACUTE TOXICITY TO *DAPHNIA MAGNA***Table 2** Cumulative Immobilisation Data in the Definitive Test

0-Hour Measured Test Concentration (mg/l)		Cumulative Immobilised Daphnia (Initial Population: 5 Per Replicate)					
		24 Hours			48 Hours		
		No. Per Replicate	Total	%	No. Per Replicate	Total	%
Control	R ₁	1			1		
	R ₂	0	1	5	0	1	5
	R ₃	0			0		
	R ₄	0			0		
0.0016	R ₁	0			0		
	R ₂	0	1	5	0	1	5
	R ₃	0			0		
	R ₄	1			1		

R₁ – R₄ = Replicates 1 to 4

ACUTE TOXICITY TO *DAPHNIA MAGNA***Table 3** Cumulative Immobilisation Data in the Positive Control

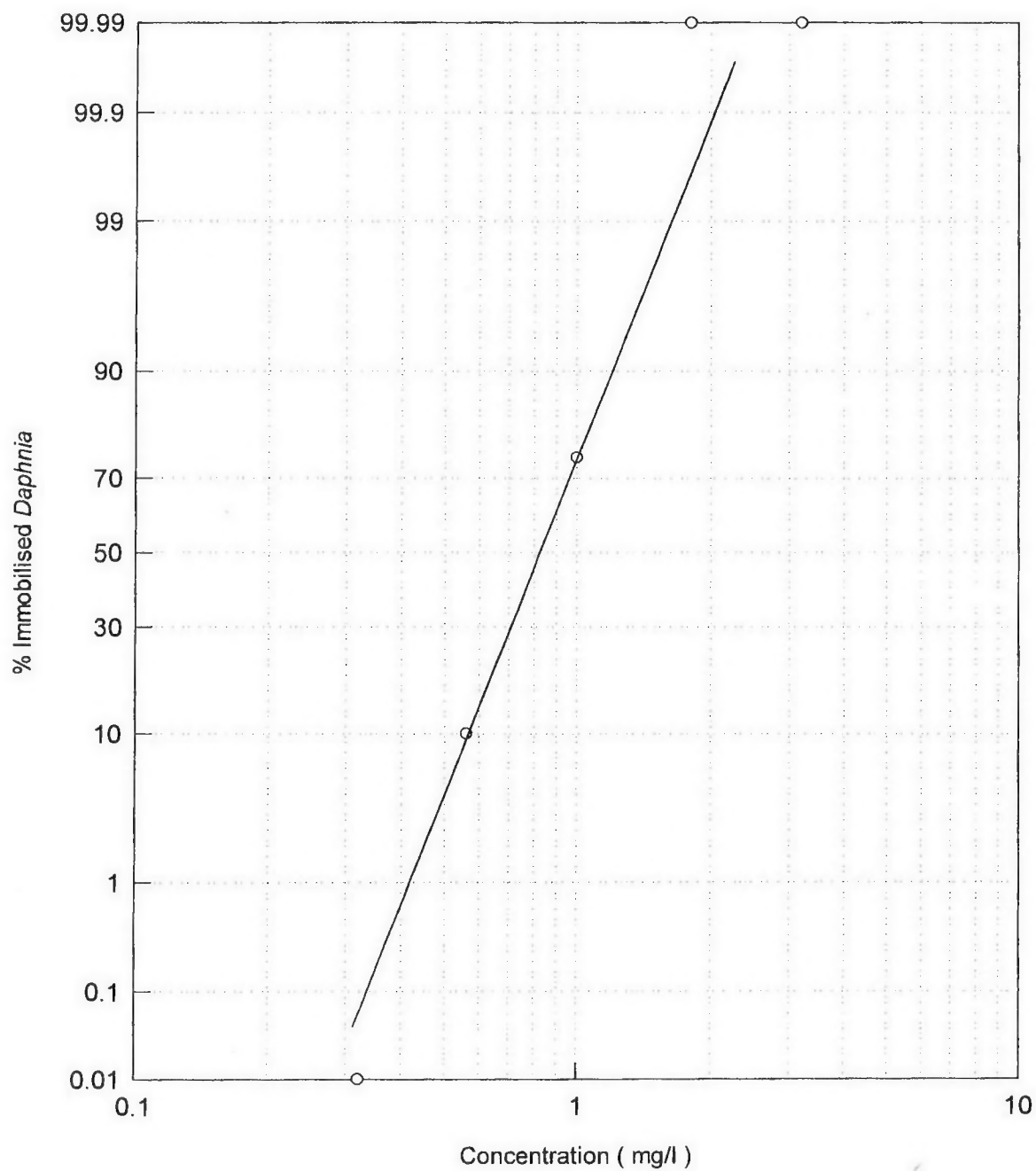
Nominal Concentration (mg/l)	Cumulative Immobilised <i>Daphnia</i> (Initial Population: 10 Per Replicate)											
	3 Hours				24 Hours				48 Hours			
	R ₁	R ₂	Total	%	R ₁	R ₂	Total	%	R ₁	R ₂	Total	%
Control	0	0	0	0	0	0	0	0	0	0	0	0
0.32	0	0	0	0	0	0	0	0	0	0	0	0
0.56	0	0	0	0	1	1	2	10	2	2	4	20
1.0	0	0	0	0	7	8	15	75	9	9	18	90
1.8	0	0	0	0	10	10	20	100	10	10	20	100
3.2	0	0	0	0	10	10	20	100	10	10	20	100

R₁ – R₂ = Replicates 1 and 2

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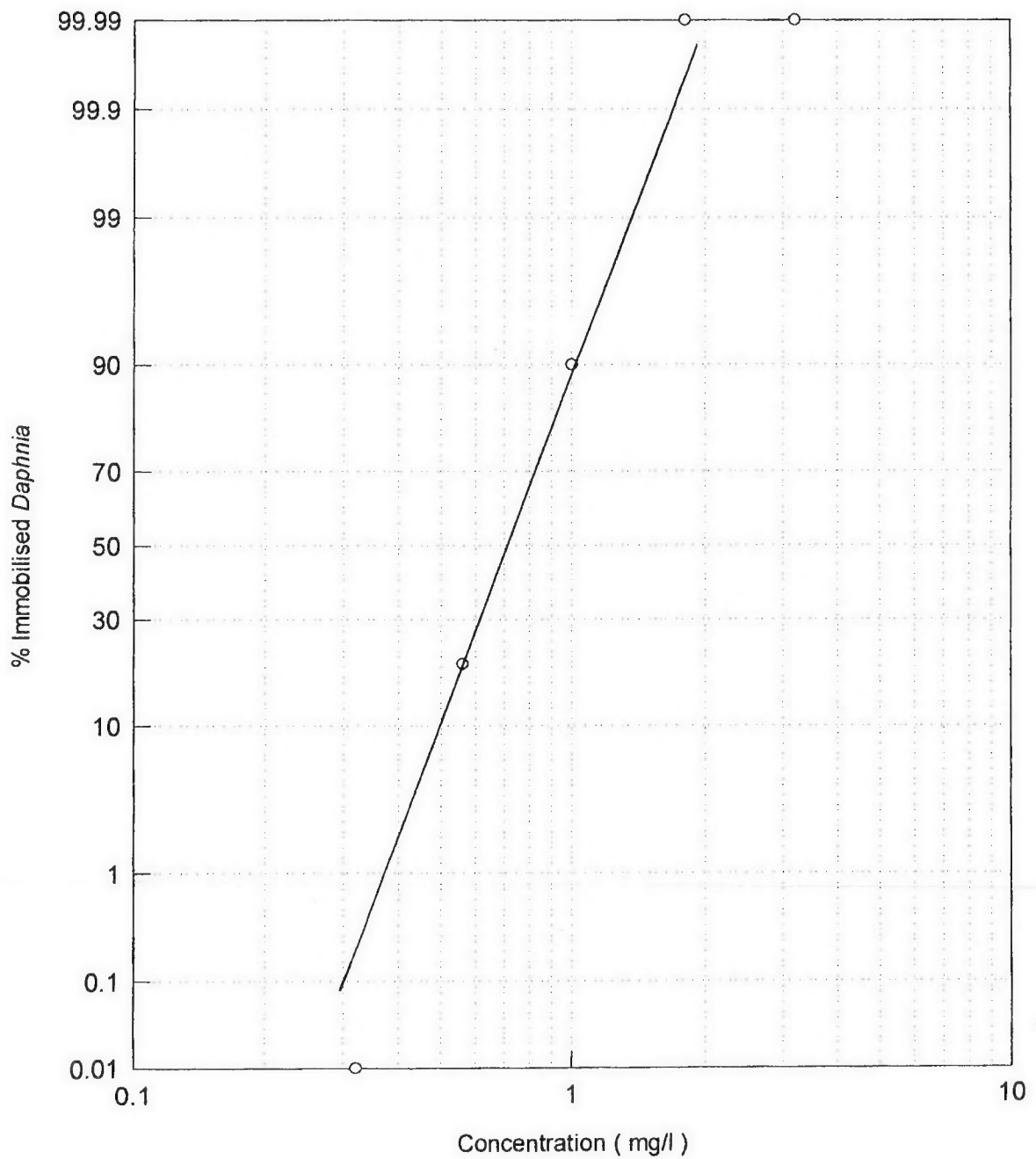
ACUTE TOXICITY TO *DAPHNIA MAGNA*

Figure 1 Concentration-Response Curve After 24 Hours in the Positive Control



ACUTE TOXICITY TO *DAPHNIA MAGNA*

Figure 2 **Concentration-Response Curve After 48 Hours in the Positive Control**



ACUTE TOXICITY TO *DAPHNIA MAGNA*

Appendix 1 Certificate of Analysis

Certificate of Analysis

1. TEST MATERIAL IDENTIFICATION

- 1) PRODUCT NAME : []
2) TEST MATERIAL(LOT No.) : 20090105
3) QUANTITY : 100g
4) CHEMICAL NAME : []
5) COMPOSITIONS OF CHEMICAL

- 6) PURITY : >99%
7) APPEARANCE : White Powder
8) MOLECULAR WEIGHT : []
9) MOLECULAR FORMULA : []

2. SOLUBILITY :

- 1) INSOLUBLE : H₂O
2) SOLUBLE : Toluene(Partially)

3. STORAGE :

- 1) Storage temperature : Room Temperature
2) Expiry date : Jan. 08. 2011

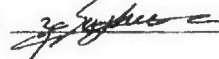
We hereby certify that the data stated here above are true and correct

Company(Manufacturer) : CHEIL INDUSTRIAL INC.
Address : (437-711) 332-2, Gocheon-dong, Uiwang-si, Gyeonggi-do, Korea

Date : Jan. 09. 2009

Name of person in charge

Sunghye Ahn



ACUTE TOXICITY TO *DAPHNIA MAGNA***Appendix 2 Reconstituted Water****i) Stock Solutions**

a) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	11.76 g/l
b) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	4.93 g/l
c) NaHCO_3	2.59 g/l
d) KCl	0.23 g/l

ii) Preparation

An aliquot (25 ml) of each of solutions a-d was added to each litre (final volume) of deionised water with a conductivity of $<5 \mu\text{S cm}^{-1}$. The reconstituted water had a pH of 7.8 ± 0.2 adjusted (if necessary) with NaOH or HCl and was aerated until the dissolved oxygen concentration was approximately air-saturation value.

The reconstituted water had an approximate theoretical total hardness of 250 mg/l as CaCO_3 .

ACUTE TOXICITY TO *DAPHNIA MAGNA***Appendix 3 Verification of Test Concentrations****1. METHOD OF ANALYSIS****1.1 Introduction**

The test material concentration in the test samples was determined by high performance liquid chromatography (HPLC) using an external standard. The test material gave a chromatographic profile consisting of a number of peaks. The results have been calculated using the main peak associated with the test material.

The method was developed by the Department of Analytical Services, Harlan Laboratories Ltd, Shardlow, UK.

1.2 Sample Preparation

A C8 (EC) solid phase extraction (SPE) cartridge (500 mg/3 ml) was sequentially pre-conditioned with methanol and water*. A volume of test sample was eluted through the cartridge and the cartridge dried. The test material was eluted from the cartridge with methanol and made to volume to give a final theoretical concentration of approximately 0.41 mg/l.

1.3 Standards

Standard solutions of test material were prepared initially in tetrahydrofuran at a nominal concentration of 1000 mg/l, these standards were then diluted using methanol to give a final theoretical concentration of 0.5 mg/l in methanol containing 10% tetrahydrofuran.

* Prepared by ELGA Purelab Option R-15 water purification

ACUTE TOXICITY TO *DAPHNIA MAGNA***Appendix 3 (continued) Verification of Test Concentrations****1.4 Procedure**

The standards and samples were analysed by HPLC using the following conditions:

HPLC System	:	Agilent Technologies 1200, incorporating autosampler and workstation
Column	:	Symmetry, C18, 3.5 μ , (50 x 3.0 mm id)
Column temperature	:	40°C
Mobile phase	:	methanol
Flow rate	:	0.8 ml/min
UV/Vis detector wavelength	:	210 nm
Injection volume	:	50 μ l
Retention time	:	approximately 1.4 minutes

2. PRE-STUDY MEDIA PREPARATION TRIAL

An amount of test material (550 mg) was dispersed, in duplicate, in 11 litres of reconstituted water. These were stirred using a propeller stirrer at approximately 1500 rpm at approximately 21°C for periods of 24 and 48 hours.

Samples were taken for analysis following removal of any undissolved test material by centrifugation at 10000 or 40000 *g* for 30 minutes or following filtration through 0.2 μ m Sartorius Sartopore filters with the first 500ml or 1 litre being discarded.

Stirring Period and Treatment	Concentration Found (mg/l)
24 Hours Control	<LOQ
24 Hours Centrifuged 10000 <i>g</i>	0.00410
24 Hours Centrifuged 40000 <i>g</i>	0.00261
24 Hours Filtered 500 ml discarded	<LOQ
24 Hours Filtered 1 litre discarded	<LOQ

LOQ = Limit of quantitation

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ACUTE TOXICITY TO *DAPHNIA MAGNA***Appendix 3 (continued) Verification of Test Concentrations**

Stirring Period and Treatment	Concentration Found (mg/l)
48 Hours Control	<LOQ
48 Hours Centrifuged 10000 g	0.00539
48 Hours Centrifuged 40000 g	0.00624
48 Hours Filtered 500 ml discarded	<LOQ
48 Hours Filtered 1 litre discarded	<LOQ

All results have been corrected for a recovery rate of 79%.

3. VALIDATION**3.1 Linearity**

A range of standard solutions covering 0.052 to 10 mg/l (exceeding the range of the working sample concentrations) was analysed.

Linearity was confirmed ($R^2 = 0.9998$) in the range 0 to 10 mg/l.

The results are presented graphically on page 30.

3.2 Recoveries

Preliminary test samples, accurately fortified at a known concentration of test material, were prepared and analysed.

The recovery samples were prepared by addition of a standard solution of test material to a sample of test medium. A standard solution was accurately prepared by dissolving the test material initially in tetrahydrofuran, then further diluted in methanol. An accurate volume of the standard solution was added to a known volume of test medium to achieve the required concentration of test material.

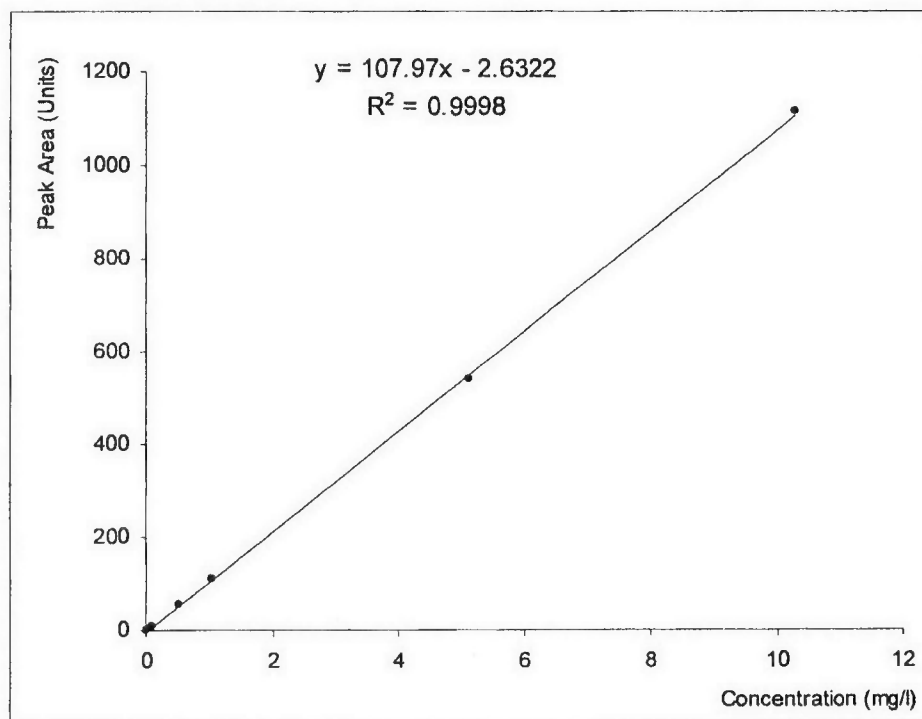
LOQ = Limit of quantitation

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ACUTE TOXICITY TO *DAPHNIA MAGNA*

Appendix 3 (continued) Verification of Test Concentrations

Linearity of Detector Response



ACUTE TOXICITY TO *DAPHNIA MAGNA*

Appendix 3 (continued) Verification of Test Concentrations

Fortification (mg/l)	Recoveries		
	(mg/l)	(%)	Mean %
0.00422	0.00321	76	77.820
0.00422	0.00351	83	
0.00422	0.00312	74	
0.00422	0.00334	79	
0.00422	0.00324	77	

The recovery results are slightly below the acceptance limits of 80 - 120% therefore all test sample results will be corrected for an overall mean recovery rate of 77.820%

The method has been considered to be sufficiently accurate for the purposes of this test.

The limit of quantitation has been assessed down to 0.000386 mg/l.

4. STABILITY

Preliminary test samples were prepared, analysed initially and then after storage in sealed glass vessels at ambient temperature in light and dark conditions for approximately 48 hours (equivalent to the test exposure period). In addition a test sample was tested for stability without prior mixing (sonication) of the test sample bottle to assess for losses due to adsorption and/or insolubility.

Nominal concentration (mg/l)	0.0041
Concentration found initially (mg/l)	0.00328
Concentration found after storage in light conditions (mg/l)	0.00345
Expressed as a percent of the initial concentration	105
Concentration found after storage in dark conditions (mg/l)	0.00343
Expressed as a percent of the initial concentration	104
Concentration found after storage in dark conditions (mg/l) – unsonicated sample	0.00366
Expressed as a percent of the initial concentration	112

ACUTE TOXICITY TO *DAPHNIA MAGNA***Appendix 3 (continued) Verification of Test Concentrations**

The test samples have been shown to be stable in the test medium.

The unsonicated stability vessel showed no evidence of insolubility or adherence to glass.

5. RESULTS

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
0 hours	Control	<LOQ	-
	0.0041 R ₁ -R ₂	0.00161	39
	0.0041 R ₃ -R ₄	0.00167	41
48 hours	Control	0.000486/<LOQ**	- / -**
	0.0041 R ₁ -R ₂	0.000514	13
	0.0041 R ₃ -R ₄	0.00106	26

6. DISCUSSION

The detection system was found to have acceptable linearity. The analytical procedure had low recoveries of test material in test medium which was overcome by correction of an overall recovery rate of 77.820%. A method of analysis was validated and proven to be suitable for use.

* Test sample concentrations corrected for an overall mean recovery rate of 77.820%

** Duplicate sample stored frozen prior to analysis

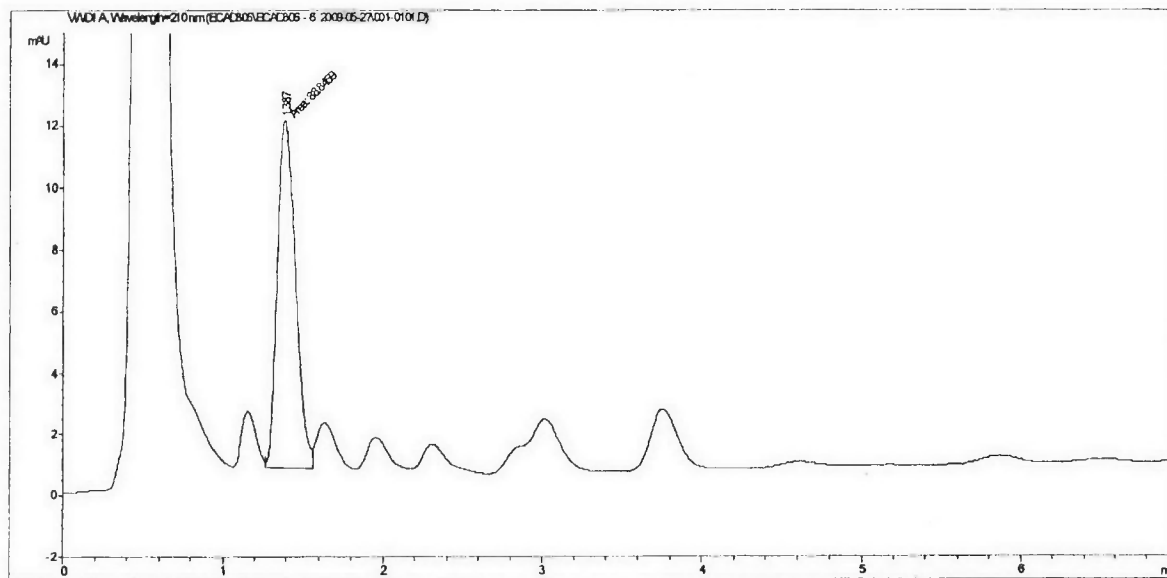
LOQ = Limit of quantitation

R₁ - R₄ = Replicates 1 to 4

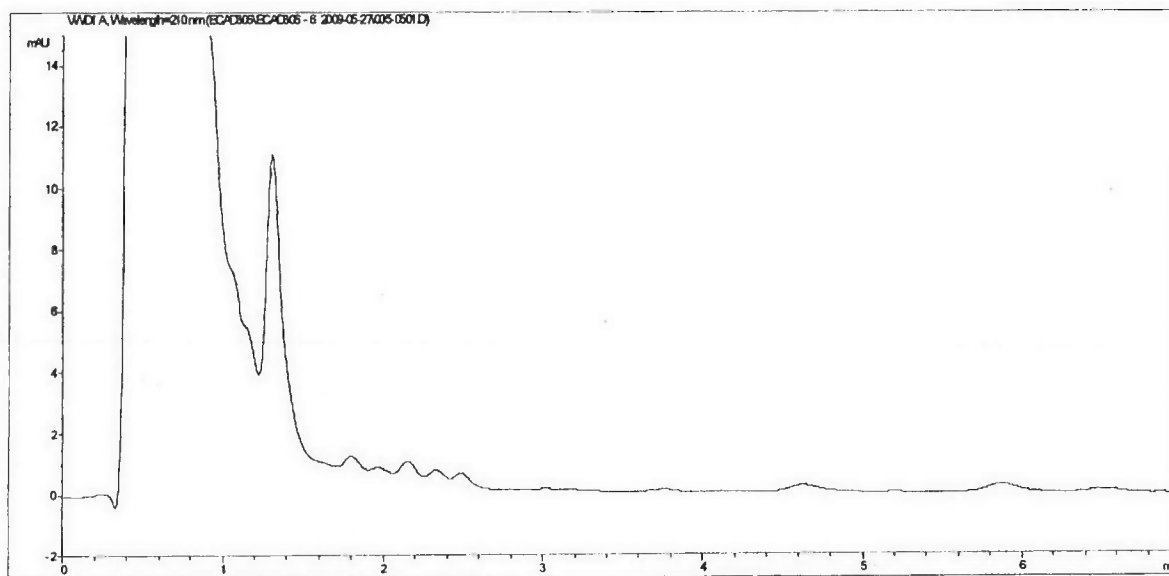
ACUTE TOXICITY TO *DAPHNIA MAGNA*

Appendix 3 (continued) Verification of Test Concentrations

7. TYPICAL CHROMATOGRAPHY



Standard 0.50 mg/l

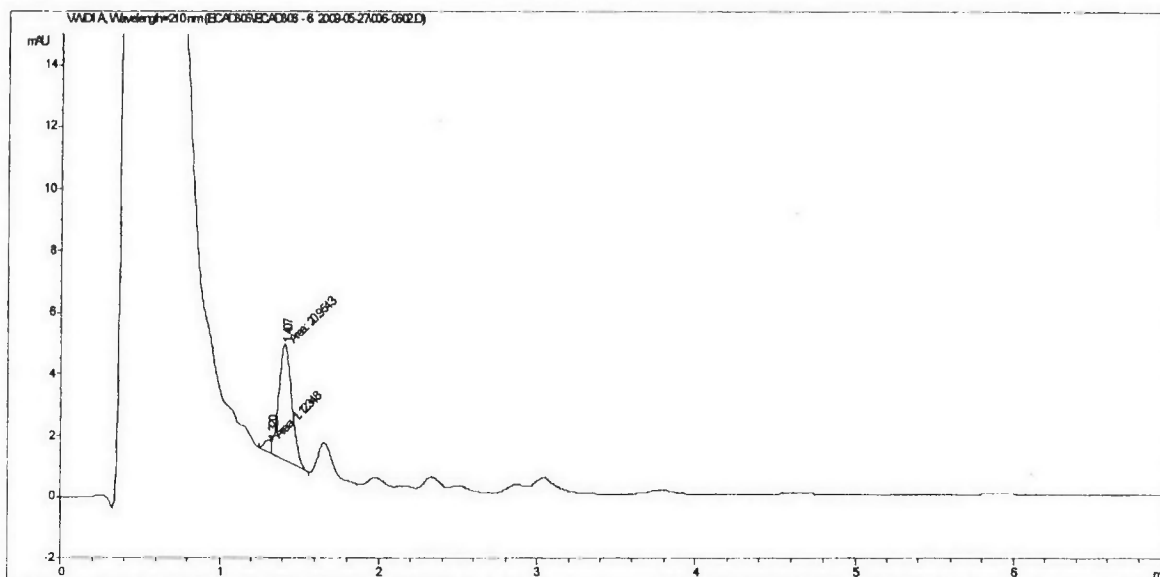
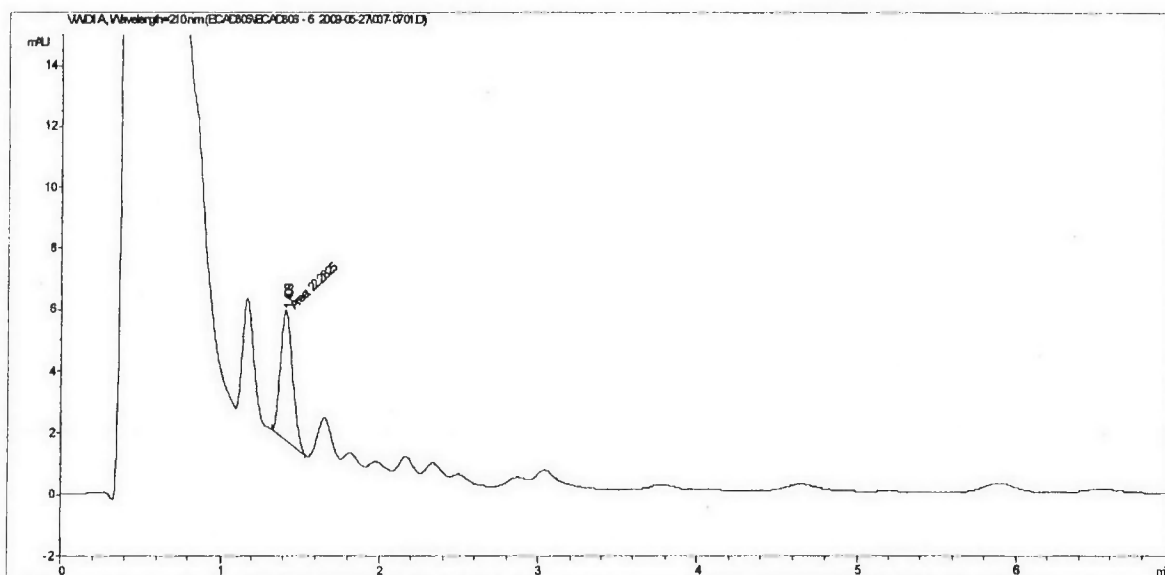


Control Sample

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ACUTE TOXICITY TO *DAPHNIA MAGNA*

Appendix 3 (continued) Verification of Test Concentrations

Test Sample 0.0041 mg/l R₁-R₂Test Sample 0.0041 mg/l R₃-R₄

R₁ - R₄ = Replicates 1 to 4

ACUTE TOXICITY TO *DAPHNIA MAGNA*

Appendix 4 Physico-Chemical Measurements

0-Hour Measured Concentration (mg/l)		0 Hours				24 Hours	48 Hours			
		pH	mg O ₂ /l	%ASV*	T°C	T°C	pH	mg O ₂ /l	%ASV*	T°C
Control	R ₁	8.0	9.0	99	20	22	8.0	8.5	98	22
	R ₂	8.0	9.0	101	21	22	8.0	8.6	99	22
	R ₃	8.0	9.0	101	21	22	8.0	8.4	97	22
	R ₄	8.0	9.0	101	21	22	8.0	8.5	98	22
0.0016	R ₁	8.0	8.1	93	22	22	8.0	8.1	91	21
	R ₂	8.0	8.1	93	22	22	8.0	8.1	93	22
	R ₃	8.0	8.1	93	22	22	8.0	8.1	91	21
	R ₄	8.0	8.1	93	22	22	8.0	8.2	94	22

*ASV = Dissolved oxygen concentration expressed as a percentage of Air Saturation Value
R₁ – R₄ = Replicates 1 to 4

**Appendix 5 Statement of GLP Compliance in Accordance with Directive
2004/9/EC**



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

A handwritten signature in black ink, appearing to read 'A. Gray', with the date '4/3/09' written below it.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



**ACTIVATED SLUDGE: RESPIRATION INHIBITION TEST**

HLS study number:	ZIE0002
Version ID:	Final Report
Issue date:	29 May 2009

Sponsor and Test Facility Details

Sponsor

Cheil Industries Inc
(437-711) 332-2 Gocheon-dong
Uiwang-si
Gyeonggi-do
KOREA

Test Facility

Huntingdon Life Sciences Ltd
Eye Research Centre
Eye
Suffolk
IP23 7PX
UK

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Compliance with Good Laboratory Practice

☐ Activated Sludge: Respiration Inhibition Test

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.


The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No. L 50/44).

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

In line with normal practice in this type of study, an assessment of the stability or homogeneity of the test substance formulation and achieved concentration was not conducted.


Robert A Dickinson BSc
Study Director
Huntingdon Life Sciences

Date

29 May 2009

Quality Assurance Statement

Activated Sludge: Respiration Inhibition Test

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	05 Mar 2009 – 10 Mar 2009	10 Mar 2009
Report Audit	17 Apr 2009	17 Apr 2009

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Dose Formulation	23 Jan 2009	23 Jan 2009
Set up of test or reference mixture	02 Dec 2008	02 Dec 2008
Environmental measurements	02 Apr 2009	07 Apr 2009

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.

S. Watts
Sarah Watts CBiol MIBiol MRQA
Lead Auditor
Department of Quality Assurance
Huntingdon Life Sciences

28 May 2009
Date

Contributing Scientists

☐ . ☐ Activated Sludge: Respiration Inhibition Test

Study Management

Aquatic Ecotoxicology and Biodegradation

Robert A Dickinson BSc
Study Director

Georgina L Podd BSc
Scientific Trainee

Summary

The effect of [] on the respiration rate of activated sludge was assessed by the methods detailed in EC Directive 88/302, Method C.11, 'Biodegradation - Activated Sludge Respiration Inhibition Test' and OECD Test Guideline 209, 'Activated Sludge, Respiration Inhibition Test'. The test procedures were also based on methods outlined in United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (OPPTS) Method 850.6800, 'Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals', Public Draft, April 1996.

Samples of activated sludge (suspended solids 1.6 g/L), fed with synthetic sewage, were exposed to dilutions of the test substance for three hours. Their rates of oxygen consumption were determined using an oxygen electrode and compared with those of controls, containing activated sludge and synthetic sewage alone, which were established at the beginning and end of the test.

The study employed nominal [] concentrations of 10, 30, 100, 300 and (triplicate) 1000 mg/L. The reference inhibitor 3,5-dichlorophenol (3,5-DCP) was employed at 3, 10 and 32 mg/L, as a positive control.

The specific respiration rate of the control culture established at the end of the test (30.0 mgO₂/g/h) was 96% of the rate established at the start (31.3 mgO₂/g/h). The three-hour 50% effect concentration (EC₅₀) for 3,5-DCP was calculated to be 6.8 mg/L (95% confidence limits, 5.8 - 7.8 mg/L). These results show that the sample of activated sludge employed was sensitive to inhibition.

[] was considered to have had no biologically significant inhibitory effect on the respiration rate of activated sludge at any of the concentrations employed in the test. The EC₂₀, EC₅₀ and EC₈₀ of the test substance could not be calculated but these must be greater than 1000 mg/L, the highest level tested.

1. Introduction

The objective of this study was to assess the effects of [] on sewage micro-organisms by measuring the rate of oxygen uptake of activated sludge at $20 \pm 2^\circ\text{C}$ in its presence at a range of concentrations.

The methods employed were designed to meet the requirements of EC Directive 88/302, Method C.11, 'Biodegradation - Activated Sludge Respiration Inhibition Test' and OECD Test Guideline 209, 'Activated Sludge, Respiration Inhibition Test'. The test procedures were also based on methods outlined in United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (OPPTS) Method 850.6800, 'Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals', Public Draft, April 1996.

The protocol was approved by the Sponsor on 9 March 2009 and by the Study Director and Huntingdon Life Sciences Management on 5 March 2009.

The experimental start and completion dates were 25 and 26 March 2009, respectively.

The test was preceded by a formulation trial.

2. Test Substance

Identity:

[]

Chemical Name:

[-]

Lot number:

20090213

CAS number:

[-]

Purity:

>99%

Expiry date:

12 February 2011

Appearance:

White powder

Storage conditions:

Room temperature

Date received:

19 February 2009

3. Experimental Procedure

3.1 Reference Inhibitor

3,5-Dichlorophenol (3,5-DCP), 99%.

3.2 Dilution Water

The dilution water used to prepare solutions of test mixtures, synthetic sewage and the reference substance was reverse osmosis (RO) water.

3.3 Synthetic Sewage

Synthetic sewage feed for activated sludge was prepared by dissolving the following in one litre of RO water:

peptone	-	16.0 g
meat extract	-	11.0 g
urea	-	3.0 g
sodium chloride	-	0.7 g
calcium chloride dihydrate	-	0.4 g
magnesium sulphate heptahydrate	-	0.2 g
di-potassium hydrogen phosphate	-	2.8 g

3.4 Preparation of the Microbial Inoculum

A sample of activated sludge was obtained the day before the start of the test from Worlingworth Sewage Treatment Works (Suffolk, UK), which treats predominantly domestic waste. In the laboratory, the sample was maintained under aerobic conditions until required. The concentration of suspended solids in a homogenised sample was determined on the day of collection and immediately before the start of the test.

On the day of collection, an aliquot (10 mL) of the activated sludge was filtered through a dried and preweighed Whatman GF/C filter paper, which was then dried again at approximately 105°C for at least one hour, allowed to cool in a desiccator and reweighed. The mixed liquor suspended solids (MLSS) content of the activated sludge was then calculated. Synthetic sewage (50 mL/L) was added to the stock of activated sludge and this was aerated overnight.

On the day of the test, the MLSS content of the sludge was determined (in triplicate) and adjusted to 4 g/L by the addition of tap water. The pH and temperature of the sludge were also measured. Aliquots (200 mL) were then added to each mixture to give a final MLSS concentration of 1.6g/L.

3.5 Preparation of Solutions of the Reference Substance (3,5-Dichlorophenol)

A concentrated solution of 3,5-DCP (500 mg/L) was prepared by dissolving 0.5 g in 10 mL of 1N sodium hydroxide and diluting to approximately 30 mL with RO water. Sulphuric acid (1N) was added to the point of incipient precipitation and the solution made up to a final volume of one litre with RO water. The pH of this solution was then measured. Nominal concentrations of 3, 10 and 32 mg/L were prepared by dilution of this concentrated solution.

3.6 Test Methods

Results of a preliminary formulation trial showed that the test substance formed a dispersion in RO water. Therefore, at test initiation appropriate weights were established in one-litre test beakers, RO water (284 mL) was added and the mixtures then treated with ultrasound for *ca.* 15 minutes in order to form dispersions. The pH of the mixtures was then determined and no adjustment was necessary. Additions of synthetic sewage and microbial inoculum were made at fifteen minute-intervals to give a final volume of 500 mL according to the following schedule:

Test mixture	Weight of test substance (mg) and volume of reference solution (mL)	Synthetic sewage (mL)	Water (mL)	Microbial inoculum (mL)
Control (1)	0	16	284	200
Test substance(mg/L)				
10	5	16	284	200
30	15	16	284	200
100	50	16	284	200
300	150	16	284	200
1000 (in triplicate)	500	16	284	200
3,5-DCP (mg/L)				
3	3	16	281	200
10	10	16	274	200
32	32	16	252	200
Control (2)	0	16	284	200

The prepared mixtures were aerated for three hours in a thermostatically-controlled water bath, using an aerator connected to a laboratory supply of oil-free compressed air (one litre/minute). Following the exposure period, a well-mixed sample of each mixture was transferred to a biochemical oxygen demand (BOD) bottle (nominal capacity, 270 mL). The rate of oxygen consumption was measured, over a period of approximately 10 minutes or until the dissolved oxygen concentration fell below 2 mgO₂/L using a Yellow Springs Instrument (YSI) dissolved oxygen meter, with temperature probe and self stirring bottle probe, connected to a chart recorder. The pH and temperature of the samples were measured at the start and end of the test.

3.7 Calculation of Results

The oxygen consumption rate of each test, reference and control mixture was calculated from oxygen levels in the following way:

$$r = \frac{DO_{(1)} - DO_{(2)}}{t}$$

where:

- r = oxygen consumption rate (mgO₂/L/minute)
- DO₍₁₎ = initial oxygen level (mgO₂/L)
- DO₍₂₎ = final oxygen level (mgO₂/L)
- t = time over which measurements were made (minutes)

The specific respiration rate of each mixture was calculated from the rate of oxygen consumption in the following way:

$$SRR = \frac{r \times 60}{MLSS}$$

where:

- SRR = specific respiration rate (mgO₂/g/h)
- MLSS = concentration of mixed liquor suspended solids in the sample of activated sludge (1.6 g/L) in the test or control mixture.

The inhibitory effect of the test or reference substance at a particular concentration was calculated by expressing the specific respiration rate (SRR) as a percentage of the mean of the respiration rates of the two controls in the following way:

$$\% \text{ inhibition} = 1 - \frac{(2 R_s)}{R_{c1} + R_{c2}} \times 100$$

where:

- R_s = rate (SRR) of oxygen consumption of test or reference substance
- R_{c1} = rate (SRR) of oxygen consumption of control 1
- R_{c2} = rate (SRR) of oxygen consumption of control 2

The EC₂₀, EC₅₀ and EC₈₀ of the test substance and EC₅₀ of the reference substance and their 95% confidence limits (Donaldson and Schnabel, 1985) were calculated using the SAFEstat curvefit programme (SAS Institute, 1999). Since the permitted maximum difference for specific respiration rates in control beakers established at the beginning and end of the test period was 15%, this value was used to define the criteria for biologically significant levels of inhibition in mixtures containing the test substance. As this was acceptably low (i.e., <15%) and variation in overall response was <20% in the mixtures that contained the test substance at 1000 mg/L, the results of this test were reported and further testing was not performed.

4. Deviations from Protocol

There were no deviations from the protocol.

5. Maintenance of Records

All raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Archive.

These records will be retained for a minimum of one year from the date of issue of the final report. At the end of the one-year retention period the Sponsor will be contacted and advice sought on their future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

6. Results

Dissolved oxygen concentrations and measurement times are given in Table 1. Temperature, pH, measurements of respiration rates and percentage inhibition are given in Table 2.

Measurements of the pH of the aqueous stock solution of the reference substance and of the sample of activated sludge (4 g/L) before the start of the test are given below.

Preparation	pH
3,5-DCP stock solution	7.7
Activated sludge	6.9

Sludge respiration rates were progressively reduced in the presence of increasing concentrations of 3,5-DCP. The three-hour 50% effect concentration (EC_{50}) for 3,5-DCP was calculated to be 6.8 mg/L (95% confidence limits, 5.8 - 7.8 mg/L).

The specific respiration rates of the control culture established at the end of the test (30.0 mgO₂/g/h) was 96% of the rate established at the start (31.3 mgO₂/g/h). These results show that the test was valid and that the sample of activated sludge employed was sensitive to inhibition.

[] was considered to have had no biologically significant inhibitory effect on the respiration rate of activated sludge at any of the concentrations employed in the test. Respiration rates were decreased by, at most, 4% in one mixture at a test concentration of 1000 mg/L. The EC_{20} , EC_{50} and EC_{80} of the test substance could not be calculated but these must be greater than 1000 mg/L, the highest level tested.

7. Conclusion

[] was considered to have had no biologically significant inhibitory effect on the respiration rate of activated sludge at any of the concentrations employed in the test. The EC_{20} , EC_{50} and EC_{80} of the test substance could not be calculated but these must be greater than 1000 mg/L, the highest level tested.

The three-hour EC_{50} for 3,5-DCP (6.8 mg/L) fulfilled the validity criterion relating to sensitivity to inhibition (acceptable EC_{50} range 5 to 30 mg/L). The validity criterion relating to the respiration rates in the control (variation not greater than 15%) was also satisfied.

8. References

SAS INSTITUTE (1999) SAS OnlineDoc© *Version Eight*. SAS Institute Inc., Cary, NC, USA.

Donaldson, J.R. and Schnabel, R.B. (1985). Computational experience with confidence regions and confidence intervals for non-linear least squares. National Bureau of Standards, Boulder, Colorado.

Table 1 Dissolved oxygen concentrations and measurement times

Test mixture	Initial DO concentration in the culture (mgO ₂ /L)	Initial measured DO concentration (mgO ₂ /L) (DO ₍₁₎)	Final measured DO concentration (mgO ₂ /L) (DO ₍₂₎)	Measurement time (minutes) (t)
Control (1)	6.9	6.5	2.5	4.8
10 (mg/L)	7.4	6.5	2.5	4.9
30	6.6	6.3	2.5	4.6
100	6.5	6.3	2.5	4.6
300	6.9	6.5	2.5	5.1
1000	6.4	6.0	2.5	4.4
1000	6.6	6.3	2.5	4.7
1000	6.9	6.5	2.5	5.1
3,5-DCP (mg/L)				
3	7.4	6.5	2.5	6.8
10	8.2	8.0	5.0	10.0
32	9.0	8.8	7.5	9.9
Control (2)	7.0	6.5	2.5	5.0

DO – Dissolved Oxygen

Table 2 Temperature, pH and measurements of respiration rate

Test mixture	Temperature (°C)		pH		Specific respiration rate mgO ₂ /g/h	% inhibition
	Initial	Final	Initial	Final		
Control (1)	19.9	19.6	7.3	7.7	31.3	-
10 (mg/L)	19.9	19.5	7.3	7.7	30.6	0
30	19.9	19.6	7.3	7.6	31.0	0
100	20.0	19.6	7.3	7.6	31.0	0
300	20.1	19.5	7.3	7.7	29.4	4
1000	20.0	19.6	7.3	7.6	29.8	3
1000	20.0	19.6	7.3	7.6	30.3	1
1000	19.9	19.5	7.3	7.7	29.4	4
3,5-DCP (mg/L)						
3	19.9	19.6	7.2	7.9	22.1	28
10	19.9	19.6	7.2	7.7	11.3	63
32	20.0	19.5	7.3	7.8	4.9	84
Control (2)	19.9	19.5	7.2	7.7	30.0	-

The data presented were calculated using unrounded values stored in the computer database. Minor numerical differences may be observed in the respiration rate calculation if rounded values are used to calculate the data. This minor discrepancy is not considered to be significant.

Appendix 1 Eye Research Centre GLP Compliance Statement 2009**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM****GOOD LABORATORY PRACTICE****STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC****TEST FACILITY**

Huntingdon Life Sciences
Eye Research Centre
Occold
Eye
Suffolk
IP23 7PX

TEST TYPE


Analytical/Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

17-19 February 2009

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


1/5/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority


MHRA

REPORT

Study Title

**DETERMINATION OF 'READY' BIODEGRADABILITY: CARBON
DIOXIDE (CO₂) EVOLUTION TEST (MODIFIED STURM TEST) OF**



Author

Ing. M.J.E. Desmares-Koopmans

Study completion date

16 February 2009

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

**NOTOX Project 489888
NOTOX Substance 190683/A**

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APPENDICES

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2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

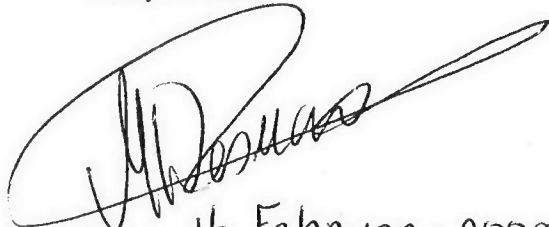
The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by NOTOX.

Analysis of stability, homogeneity and concentration of the test substance under test conditions was not performed as part of this study.

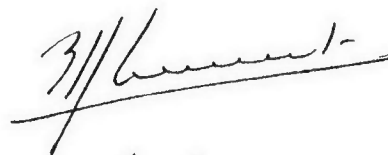
NOTOX B.V.

Ing. M.J.E. Desmares-Koopmans
Study Director



Date: 16 February 2009

Ing. E.J. van de Waart, M.Sc.
Head of In Vitro & Environmental Toxicology



Date: 16/02/2009

3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.

During the on-site process inspections procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	09-Dec-08	09-Dec-08	09-Dec-08
	Amendment 1 of protocol	30-Jan-09	30-Jan-09	30-Jan-09
	Report	11-Feb-09	11-Feb-09	11-Feb-09
Process	Environmental toxicology	26-Jan-09	05-Feb-09	05-Feb-09
	Test substance handling			
	Exposure			
	Observations/Measurements			

Head of Quality Assurance
C.J.Mitchell B.Sc.



Date: 17/2/09

4. SUMMARY

Determination of 'ready' biodegradability: carbon dioxide (CO₂) evolution test (modified Sturm test) with []

The study procedure described in this report were based on the OECD guideline No. 301 B, 1992. In addition, the procedures were designed to meet the test methods of the Commission Regulation (EC) No 440/2008 of 30 May 2008, Part C.4-C and the ISO International Standard 9439, 1999.

[] was a white powder with a purity of >99%. [] was tested in duplicate at 88 mg per 2 litres, corresponding to 10 mg TOC/l. The organic carbon content was based on the molecular formula. The Theoretical CO₂ production (ThCO₂) of [] was calculated to be 0.84 mg CO₂/mg.

The study consisted of six bottles:

- 2 blank controls (no test material),
- 2 test bottles []
- 1 positive control (sodium acetate) and
- 1 toxicity control [] plus sodium acetate).

Since [] was not sufficiently soluble to allow preparation of an aqueous solution at a concentration of 1 g/l, weighed amounts of [] were added to the test bottles (2 litres) containing medium with microbial organisms and mineral components. To this end, 10 ml of Milli-RO water was added to each weighing bottle containing the test substance. After vigorous mixing (vortex) the resulting suspension was added quantitatively to the test medium. The test solutions were continuously stirred during the test, to ensure optimal contact between the test substance and the test organisms. Test duration was 28 days (last CO₂-measurement on the 29th day).

The relative biodegradation values calculated from the measurements performed during the test period revealed no significant biodegradation of [].
In the toxicity control, [] was found not to inhibit microbial activity.

Since all criteria for acceptability of the test were met, this study was considered to be valid.

In conclusion, [] is designated as not readily biodegradable.

5. INTRODUCTION

5.1. Preface

Sponsor	Cheil Industrial Co., Ltd. Mr. Sunghee Ahn 332-2 Gocheon-dong Uiwang-si 437-711, GYEONGGI-DO Korea
Study Monitor	Chemtopia Co., Ltd. Ms. Hana Lim No. 1407 Leaders Building 1599-11 Seocho-dong, Seocho-gu 137-876 SEOUL Korea
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ing. M.J.E. Desmares-Koopmans
Technical Coordinator	J.H.J.W. Kluytmans
Study Plan	Start : 07 January 2009 Completion : 05 February 2009

5.2. Aim of the study

The purpose of the study was to evaluate a non-volatile test substance for its ready biodegradability in an aerobic aqueous medium with microbial activity introduced by inoculation with the supernatant of activated sludge.

5.3. Guidelines

The study procedures described in this report were based on the Organization for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, Section 3, Degradation and Accumulation, guideline No. 301 B: "Ready Biodegradability: CO₂ Evolution Test" adopted July 17, 1992.

In addition, the procedures were designed to meet the test methods prescribed by the following guidelines:

- Commission Regulation (EC) No 440/2008 of 30 May 2008, Part C: Methods for the determination of ecotoxicity, Publication No. L142, C.4. "Biodegradation: determination of the 'ready' biodegradability, C.4-C: Carbon dioxide (CO₂) evolution test (Modified Sturm Test).
- ISO Standard 9439 "Water Quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium - carbon dioxide evolution test (1999).

5.4. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens (except specimens requiring refrigeration or freezing) and the final report are retained in the NOTOX archives for a period of at least 2 years after finalization of the report. After this period, the

sponsor will be contacted to determine how the records and materials should be handled. NOTOX will retain information concerning decisions made.

Those specimens requiring refrigeration or freezing will be retained by NOTOX for as long as the quality of the specimens permits evaluation but no longer than three months after finalization of the report.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

5.5. Definitions

Readily biodegradable are those test substances giving a result of at least 60% biodegradation within 28 days. This pass level must be reached within the 10 days immediately following the attainment of 10% biodegradation (10-day window).

Theoretical carbon dioxide (ThCO₂) is the quantity of carbon dioxide calculated (mg) to be produced from the known or measured carbon content of the test substance when fully mineralized; also expressed as mg carbon dioxide evolved per mg test substance.

Total Organic Carbon (TOC) of a sample is the sum of the organic carbon in solution and in suspension.

6. MATERIALS AND METHODS

6.1. Test Substance

6.1.1. Test substance information

Identification	
Chemical name	
Molecular formula	
Molecular weight	
CAS Number	
Description	White powder
Batch	20081010
Purity	>99%
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	08 January 2011

6.1.2. Study specific test substance information

Stability in water	Not indicated
Solubility in water	No

6.1.3. Test concentration and preparation of test solutions

[] as a white powder with a purity of >99% [] was tested in duplicate at 88 mg per 2 litres, corresponding to 10 mg TOC/l. The organic carbon content was based on the molecular formula.

Since [] was not sufficiently soluble to allow preparation of an aqueous solution at a concentration of 1 g/l, weighed amounts of [] were added to the test bottles (2 litres) containing medium with microbial organisms and mineral components (test substance bottle A:

87.7 mg; test substance bottle B: 87.9 mg and toxicity control bottle: 87.9 mg). To this end, 10 ml of Milli-RO water was added to each weighing bottle containing the test substance. After vigorous mixing (vortex) the resulting suspension was added quantitatively to the test medium. The test solutions were continuously stirred during the test, to ensure optimal contact between the test substance and the test organisms.

6.2. Reference substance

6.2.1. Reference substance

Identification number	RS186
Name	Sodium acetate
Description	White powder (determined at NOTOX)
Molecular formula	CH ₃ COONa (taken from label)
Molecular weight	82.03 (taken from label)
Batch number	K34333668
Article number	1.06268.0250
Purity	≥99.0%
Expiry Date	28 February 2010
Certified	Yes
Storage conditions	At room temperature in the dark
Supplier	Merck, Darmstadt, Germany

6.2.2. Reference substance concentration and preparation of test solutions

A solution of sodium acetate was prepared by dissolving 402.0 mg in Milli-RO water and making this up to a total volume of 100 ml. Volumes of 20 ml from this stock solution were added to 2 litres of the test medium of the positive control bottle and the toxicity control bottle, resulting in a final concentration of 40 mg sodium acetate per litre (12 mg TOC/l).

6.3. Test system

Source	The source of test organisms was activated sludge freshly obtained from a municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, The Netherlands, receiving predominantly domestic sewage.
Treatment	The freshly obtained sludge was kept under continuous aeration until further treatment. The concentration of suspended solids was 4.7 g/l in the concentrated sludge (information obtained from the municipal sewage treatment plant). Before use, the sludge was allowed to settle (52 minutes) and the liquid was decanted for use as inoculum at the amount of 10 ml/l of mineral medium.
Reason for selection	The test has been accepted internationally (EC, OECD) for determining the 'ready' biodegradability of test substances under aerobic conditions.

6.4. Test procedure and conditions

Test duration	28 days (last CO ₂ -measurement on the 29 th day). During the test period the test media were aerated and stirred continuously.
Test vessels	2 litre all-glass brown coloured bottles.
Milli-RO / Milli-Q water	Tap-water purified by reverse osmosis (Milli-RO) and subsequently passed over activated carbon and ion-exchange cartridges (Milli-Q) (Millipore Corp., Bedford, Mass., USA).
Stock solutions of mineral components	A) 8.50 g KH ₂ PO ₄ 21.75 g K ₂ HPO ₄ 67.20 g Na ₂ HPO ₄ · 12H ₂ O 0.50 g NH ₄ Cl dissolved in Milli-Q water and made up to 1 litre, pH 7.4 ± 0.2 B) 22.50 g MgSO ₄ · 7H ₂ O dissolved in Milli-Q water and made up to 1 litre. C) 36.40 g CaCl ₂ · 2H ₂ O dissolved in Milli-Q water and made up to 1 litre. D) 0.25 g FeCl ₃ · 6H ₂ O dissolved in Milli-Q water and made up to 1 litre.
Mineral medium	1 litre mineral medium contains: 10 ml of solution (A), 1 ml of solutions (B) to (D) and Milli-RO water.
Barium hydroxide	0.0125 M Ba(OH) ₂ (Boom, The Netherlands), stored in a sealed vessel to prevent absorption of CO ₂ from the air.
Synthetic air (CO ₂ < 1 ppm)	A mixture of oxygen (ca. 20%) and nitrogen (ca. 80%) was passed through a bottle, containing 0.5 - 1 litre 0.0125 M Ba(OH) ₂ solution to trap CO ₂ which might be present in small amounts. The synthetic air was sparged through the scrubbing solutions at a rate of approximately 1-2 bubbles per second (ca. 30-100 ml/min).

6.4.1. Preparation of bottles

Pre-incubation medium	Mineral components, Milli-RO water (ca. 80% total volume) and inoculum (1% final volume) were added to each bottle. This mixture was aerated with synthetic air overnight to purge the system of CO ₂ .
Type and number of bottles	Test suspension: containing test substance and inoculum (2 bottles). Inoculum blank: containing only inoculum (2 bottles) Positive control: containing reference substance and inoculum (1 bottle). Toxicity control: containing test substance, reference substance and inoculum (1 bottle).



Preparation

The test substance and positive control were added to the bottles containing the microbial organisms and mineral components (ca. 80% of total volume). The volumes of suspensions were made up to 2 litres with Milli-RO water, resulting in the mineral medium described before.

Three CO₂-absorbers (bottles filled with 100 ml 0.0125 M Ba(OH)₂) were connected in series to the exit air line of each test bottle.

6.4.2. Determination of CO₂

Experimental CO₂ production

The CO₂ produced in each test bottle reacted with the barium hydroxide in the gas scrubbing bottle and precipitated out as barium carbonate. The amount of CO₂ produced was determined by titrating the remaining Ba(OH)₂ with 0.05 M standardized HCl (1:20 dilution from 1 M HCl (Titrisol® ampul), Merck, Darmstadt, Germany).

Measurements

Titration was made every second or third day during the first 10 days, and thereafter at least every fifth day until the 28th day, for the inoculum blank and test suspension. Titrations for the positive and toxicity control were made at least 14 days.

Each time the CO₂-absorber nearest to the test bottle was removed for titration; each of the remaining two absorbers was moved one position in the direction of the test bottle. A new CO₂-absorber was placed at the far end of the series. Phenolphthalein (1% solution in ethanol, Merck, Darmstadt, Germany) was used as pH-indicator.

On the 28th day, the pH of all test suspensions was measured and 1 ml of concentrated HCl (37%, Merck, Darmstadt, Germany) was added to the bottles of the inoculum blank and test suspension. The bottles were aerated overnight to drive off CO₂ present in the test suspension. The final titration was made on day 29.

Theoretical CO₂ production

The theoretical CO₂ production was calculated from the molecular formula.

6.4.3. Measurements and recording

pH

At the start of the test and on the 28th day.

Temperature of medium

Continuously in a vessel with Milli-RO water in the same room.

6.5. Electronic data capture

Observations/measurements in the study were recorded electronically using the following programme:

REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA)

6.6. Interpretation

6.6.1. Data evaluation

ThCO₂, expressed as mg CO₂/mg test substance, that can be generated by a test substance was calculated as follows:

$$\text{ThCO}_2 = \frac{\text{No. of carbon atoms in test substance} \times \text{Molecular weight CO}_2}{\text{Molecular weight test substance}}$$

The first step in calculating the amount of CO₂ produced is to correct for background (endogenous) CO₂ production. Thus the amount of CO₂ produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank Ba(OH)₂ traps.

The amount of 0.05 N HCl titrated is converted into mg of CO₂ produced:

$$\text{mg CO}_2 = \frac{0.05 \times \Delta \text{ ml HCl titrated}}{2} \times 44 = 1.1 \times \Delta \text{ ml HCl titrated}$$

Relative biodegradation values were calculated from the cumulative CO₂ production relative to the total expected CO₂ production based on the total carbon content of the amount of test material present in the test bottles. A figure of more than 10% biodegradation was considered as significant.

The relative biodegradation values were plotted versus time together with the relative biodegradation of the positive control. If applicable, the number of days is calculated from the attainment of 10% biodegradation until 60% biodegradation. Should this period be ≤ 10 days (10-day window), then the test substance is designated as readily biodegradable.

Toxicity control: if less than 25% biodegradation (based on ThCO₂) occurred within 14 days, the test substance was assumed to be inhibitory.

The total CO₂ evolution in the inoculum blank was determined by the cumulative difference (in ml of titrant) between the blank Ba(OH)₂ traps and fresh Ba(OH)₂.

6.6.2. Acceptability of the test

1. The positive control substance was biodegraded by at least 60% (73%) within 14 days.
2. The difference of duplicate values for %-degradation of [] was always less than 20.
3. The total CO₂ release in the blank at the end of the test did not exceed 40 mg/l (49.8 mg CO₂ per 2 litres of medium, corresponding to 24.9 mg/l).
4. The Inorganic Carbon content (IC) of the test substance (suspension) in the mineral medium at the beginning of the test was less than 5% of the Total Carbon content (TC). Since the test medium was prepared in tap-water purified by reverse osmosis (Milli-RO water (Millipore Corp., Bedford, Mass., USA, carbon levels < 500 ppb)), IC was less than 5% of TC (mainly coming from the test substance, 12 mg TOC/l).

Since all criteria for acceptability of the test were met, this study was considered to be valid.

6.7. List of deviations

6.7.1. List of protocol deviations

There were no deviations from the protocol.

6.7.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

7. RESULTS

7.1. Theoretical CO₂ production

The ThCO₂ of [] was calculated to be 0.84 mg CO₂/mg.
The ThCO₂ of Sodium Acetate was calculated to be 1.07 mg CO₂/mg.
The ThCO₂ per test bottle are given in Tables 2-5 (Appendix I).

7.2. Biodegradation

All data are included in Appendix I. The results of CO₂ production and biodegradation in each test bottle are listed in Tables 1 to 5. Table 6 contains the comparison of biodegradation of [] in bottles A and B. Figure 1 shows the curves for biodegradation of the two bottles with [] the positive control and the toxicity control.

The relative biodegradation values calculated from the measurements performed during the test period revealed no significant biodegradation of [].

In the toxicity control more than 25% biodegradation occurred within 14 days (30%, based on ThCO₂). Therefore, the test substance was assumed not to inhibit microbial activity.

7.3. Monitoring of temperature and pH

The temperature recorded in a vessel with water in the same room varied between 21.4 and 22.3°C.

The pH values of the different test media are presented in Table 7.

8. CONCLUSION

[] was not readily biodegradable under the conditions of the modified Sturm test presently performed.

APPENDIX I TABLES AND FIGURES

Table 1 CO₂ production in the blank.

Day	HCl (0.05 N) titrated (ml)		Produced CO ₂ (ml HCl)	Produced CO ₂ (mg)	Cumulative CO ₂ (mg)
	Ba(OH) ₂ ¹⁾	Blank (mean)			
2	46.98	44.65	2.33	2.6	2.6
5	46.92	43.45	3.48	3.8	6.4
7	46.84	43.34	3.50	3.9	10.2
9	48.86	44.41	4.46	4.9	15.1
14	48.10	43.35	4.75	5.2	20.4
19	46.61	40.89	5.72	6.3	26.6
23	43.91	42.34	1.57	1.7	28.4
27	47.56	40.74	6.82	7.5	35.9
29	48.60	40.61	7.99	8.8	44.7
29	48.30	45.64	2.66	2.9	47.6
29	47.61	45.57	2.04	2.2	49.8

¹⁾: "Strength" of untreated 0.0125 M Ba(OH)₂ solution (mean value)

Table 2 CO₂ production and percentage biodegradation of the positive control substance.

Day	HCl (0.05 N) titrated (ml)		Produced CO ₂ (ml HCl)	Produced CO ₂ (mg)	Cumulative CO ₂ (mg)	Biodegradation ¹⁾ (%)
	Blank (mean)	Positive control				
2	44.65	36.86	7.79	8.6	8.6	10
5	43.45	17.89	25.56	28.1	36.7	43
7	43.34	29.65	13.69	15.1	51.7	60
9	44.41	39.96	4.45	4.9	56.6	66
14	43.35	38.08	5.27	5.8	62.4	73

¹⁾: Calculated as the ratio between CO₂ produced (cumulative) and the ThCO₂ of sodium acetate: 86.0 mg CO₂/2l

Table 3 CO₂ production and percentage biodegradation of the test substance (bottle A).

Day	HCl (0.05 N) titrated (ml)		Produced CO ₂ (ml HCl)	Produced CO ₂ (mg)	Cumulative CO ₂ (mg)	Biodegradation ¹⁾ (%)
	Blank (mean)	bottle A				
2	44.65	44.81	0.00	0.0	0.0	0
5	43.45	43.83	0.00	0.0	0.0	0
7	43.34	43.38	0.00	0.0	0.0	0
9	44.41	44.38	0.02	0.0	0.0	0
14	43.35	44.24	0.00	0.0	0.0	0
19	40.89	42.11	0.00	0.0	0.0	0
23	42.34	42.98	0.00	0.0	0.0	0
27	40.74	41.74	0.00	0.0	0.0	0
29	40.61	45.41	0.00	0.0	0.0	0
29	45.64	45.30	0.34	0.4	0.4	1
29	45.57	46.63	0.00	0.0	0.4	1

¹⁾: Calculated as the ratio between CO₂ produced (cumulative) and the ThCO₂ of the test substance: 73.7 mg CO₂/2l

APPENDIX I TABLES AND FIGURES – CONTINUED

Table 4 CO₂ production and percentage biodegradation of the test substance (bottle B).

Day	HCl (0.05 N) titrated (ml)		Produced CO ₂ (ml HCl)	Produced CO ₂ (mg)	Cumulative CO ₂ (mg)	Biodegradation ¹⁾ (%)
	Blank (mean)	bottle B				
2	44.65	44.41	0.24	0.3	0.3	0
5	43.45	43.58	0.00	0.0	0.3	0
7	43.34	43.20	0.13	0.1	0.4	1
9	44.41	44.85	0.00	0.0	0.4	1
14	43.35	44.76	0.00	0.0	0.4	1
19	40.89	42.93	0.00	0.0	0.4	1
23	42.34	42.33	0.01	0.0	0.4	1
27	40.74	42.21	0.00	0.0	0.4	1
29	40.61	42.58	0.00	0.0	0.4	1
29	45.64	45.16	0.48	0.5	1.0	1
29	45.57	46.61	0.00	0.0	1.0	1

¹⁾: Calculated as the ratio between CO₂ produced (cumulative) and the ThCO₂ of the test substance: 73.8 mg CO₂/2l

Table 5 CO₂ production and percentage biodegradation of the toxicity control.

Day	HCl (0.05 N) titrated (ml)		Produced CO ₂ (ml HCl)	Produced CO ₂ (mg)	Cumulative CO ₂ (mg)	Biodegradation ¹⁾ (%)
	Blank (mean)	toxicity control				
2	44.65	43.22	1.43	1.6	1.6	1
5	43.45	22.11	21.34	23.5	25.0	16
7	43.34	33.58	9.76	10.7	35.8	22
9	44.41	38.71	5.70	6.3	42.0	26
14	43.35	38.57	4.78	5.3	47.3	30

¹⁾: Calculated as the ratio between CO₂ produced (cumulative) and the sum of the ThCO₂ of the test substance and positive control: 159.9 mg CO₂/2l

ThCO₂ test substance: 73.8 mg CO₂/2l

ThCO₂ sodium acetate: 86.0 mg CO₂/2l

Table 6 Comparison of biodegradation of the test substance in bottles A and B.

Day	Biodegradation (%)			
	Bottle A	Bottle B	Mean A and B	Δ A-B ¹⁾
2	0	0	0	0
5	0	0	0	0
7	0	1	0	1
9	0	1	0	1
14	0	1	0	1
19	0	1	0	1
23	0	1	0	1
27	0	1	0	1
29	0	1	0	1
29	1	1	1	1
29	1	1	1	1

¹⁾: Absolute difference in biodegradation between bottles A and B

APPENDIX I TABLES AND FIGURES – CONTINUED

Table 7 pH values of different test media.

Test medium:	Just before the start of the test:	On day 28:
Blank control (A)	7.6	7.6
Blank control (B)	7.6	7.6
Positive control	7.6	7.9
SH-1 (A)	7.6	7.6
SH-1 (B)	7.6	7.6
Toxicity control	7.6	7.9

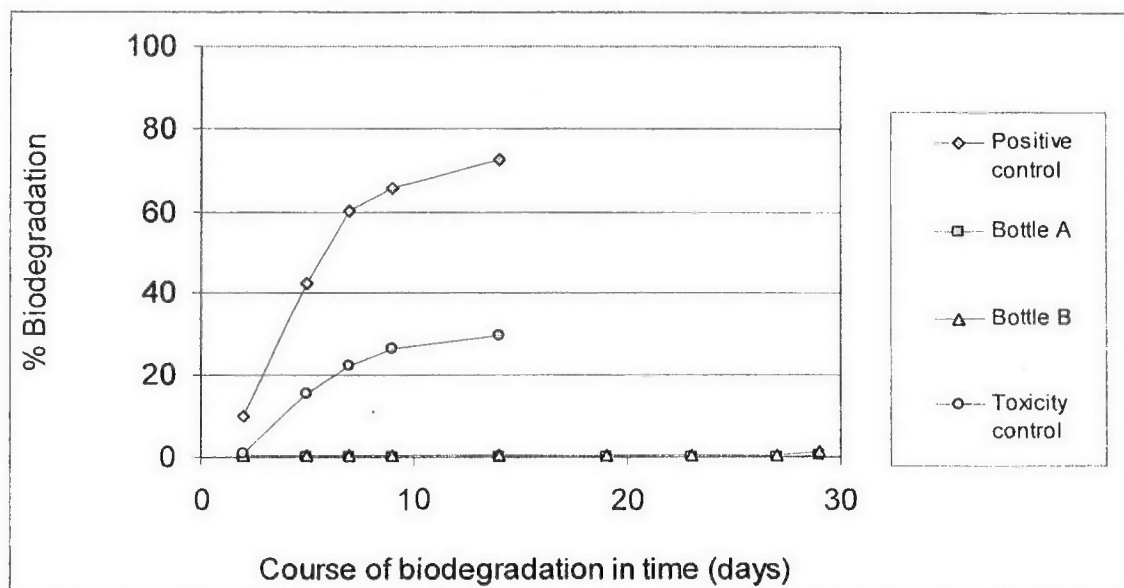


Figure 1 Biodegradation of [] and sodium acetate in the modified Sturm test



Soil Adsorption

HLS study number:	ZIE0001
Version ID:	Final
Issue date:	3 June 2009

Sponsor and Test Facility

Sponsor	Cheil Industries Inc. (437-711) 332-2 Gocheon-dong Uiwang-si Gyeonggi-do KOREA
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Test facility	Huntingdon Life Sciences Eye Research Centre Eye Suffolk IP23 7PX UK
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[] Soil adsorption

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

EC Commission Directive 2004/10/EC of 11 February 2004.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

P. Sydney

P Sydney BSc MSc
Study Director
Huntingdon Life Sciences Ltd

3 June 2009

Date

Soil adsorption

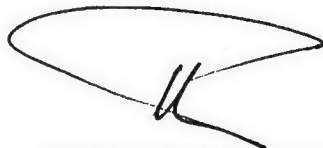
The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	18 Mar 2009	18 Mar 2009
Report Audit	15 Apr 2009	15 Apr 2009

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Chromatography	9 Jan 2009	9 Jan 2009
Standard/sample preparation	9 Feb 2009	9 Feb 2009

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were promptly reported to Company Management.



T Pullinger MIAT MRQA
Group Manager
Department of Quality Assurance
Huntingdon Life Sciences Ltd

03 JUNE 2009

Date

Contributing Scientists

[]: Soil adsorption

Study management

P Sydney BSc MSc
Study Director
Product Chemistry

Other responsible staff

L McLeay MChem
Scientist
Product Chemistry

Summary

A study was performed to estimate the adsorption coefficient (K_{oc}) of [] by HPLC. The method followed is amongst those described in the Annex to EEC Directive 92/69/EEC (Method C19) and the OECD Guidelines for the Testing of Chemicals (Method 121).

[] was determined to have a $\log_{10}K_{oc}$ value of greater than 5.6 ($K_{oc} > 4 \times 10^5$).

1. Introduction

1.1 Objective

A study was performed to estimate the adsorption coefficient (K_{oc}) of [] by HPLC.

1.2 Regulatory compliance

The method followed is amongst those described in the Annex to EEC Directive 92/69/EEC (Method C19) and the OECD Guidelines for the Testing of Chemicals (Method 121).

1.3 Study schedule

Study initiation date	: 11 March 2009
Experimental start date	: 1 April 2009
Experimental completion date	: 3 April 2009
Study completion date	: 3 June 2009

1.4 Maintenance of records

Primary data from the test, and a copy of the final report, are stored in the archives of Huntingdon Life Sciences.

2. Test Substance

Identity:

[.]

Chemical name:

[.]

Appearance:

White powder

Storage conditions:

Room temperature

Lot number:

20090213

Expiry date:

12 February 2011

Purity:

>99%

Date received:

19 February 2009

3. Soil Adsorption (EEC Method C19, OECD Method 121)

3.1 Definition and units

The adsorption coefficient is defined as the ratio between the concentration of the substance in the soil and the concentration of the substance in the aqueous phase, both measured when adsorption equilibrium is reached.

The adsorption coefficient is usually given in the form of its logarithm to base ten ($\log_{10}K_{oc}$).

3.2 Method

The sorption behaviour of the test substance on soil can be investigated using high performance liquid chromatography (HPLC). This is performed on commercially available analytical columns packed with a solid phase containing a moderately polar stationary phase with lipophilic and polar moieties (e.g. cyano-propyl bonded phase). The chemicals injected onto the column move along it by partitioning between the mobile phase and the stationary phase. The velocity of each component thereby depends on the degree of adsorption on the stationary phase. The dual nature of the stationary phase allows for interaction of polar and apolar parts of a molecule in a similar way as is the case for soil. This enables the relationship between the retention time on such a column and the adsorption coefficient on the organic parts of the soil to be established.

The adsorption coefficient is deduced from the capacity factor (k) given by:

$$k = \frac{t_r - t_o}{t_o}$$

where t_r is the measured retention time of sample or reference
and t_o is the retention time of the internal deadtime standard

3.3 Procedure

Preliminary work indicated that [] would have a high $\log_{10}K_{oc}$ value and the following limit test was thus performed:

A solution of [] (28 mg/l in methanol:tetrahydrofuran (9:1 v/v)) was prepared and chromatographed alongside the reference substance DDT, which has a known $\log_{10}K_{oc}$ value of 5.6*.

*OECD Method 121.

3.4 HPLC conditions

Instrument:	Hewlett Packard 1200 Series Liquid Chromatograph
Column:	Hypersil CPS (25 cm x 4.6 mm internal diameter)
Column temperature:	25°C
Mobile phase composition:	Methanol:water (55:45 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl (DDT); 100 µl (SH-1)
Detector:	UV set at 210 nm

3.5 Results

Figures 1 to 3 present examples of chromatograms generated during this test. It was evident that the retention time of [] was longer than that of DDT, the highest available reference standard recommended by the test guideline, indicating a $\log_{10}K_{oc}$ value of greater than 5.6 ($K_{oc} > 4 \times 10^5$).

3.6 Conclusion

[] was determined to have a $\log_{10}K_{oc}$ value of greater than 5.6 ($K_{oc} > 4 \times 10^5$).

Figure 1 Chromatogram of DDT

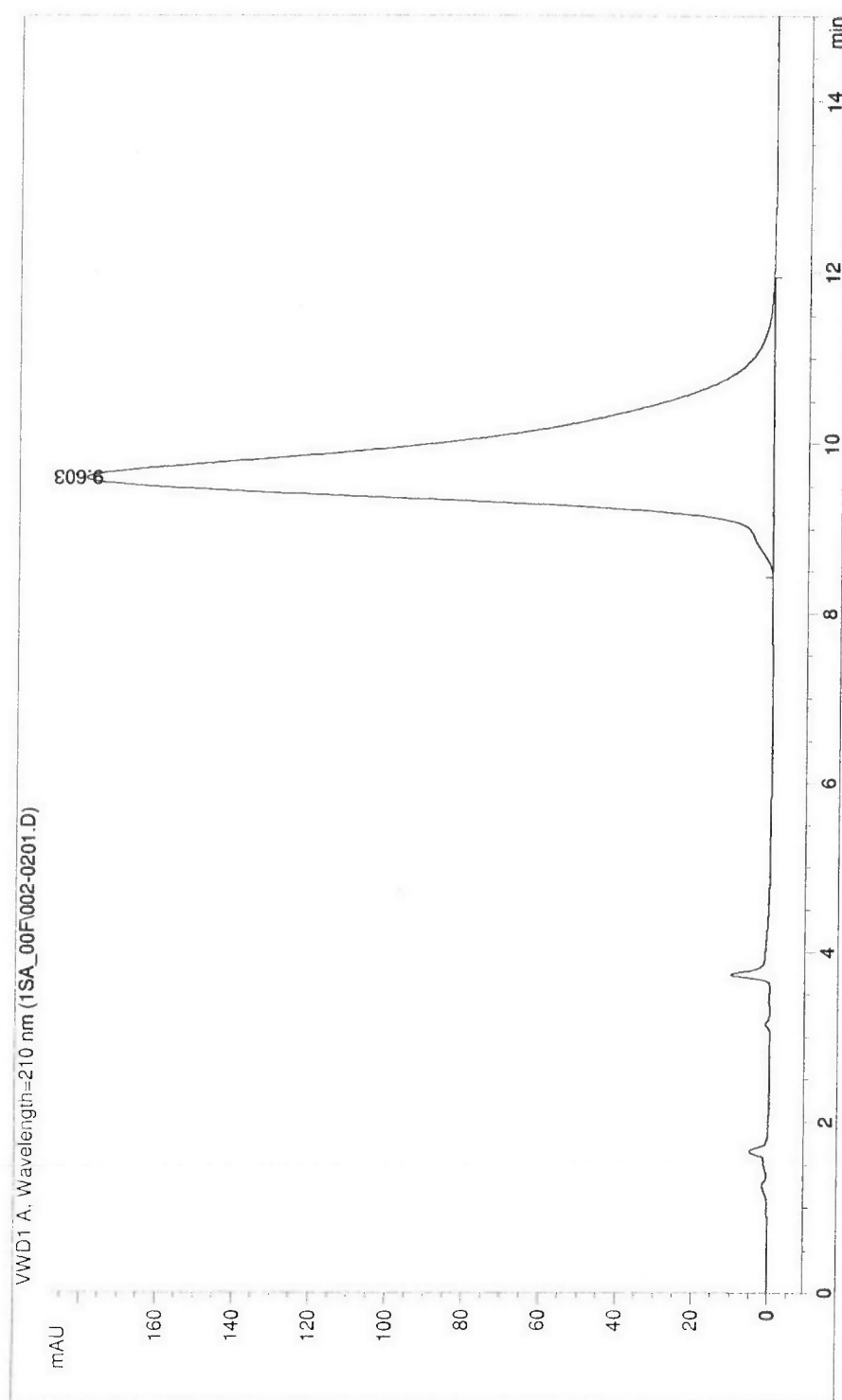


Figure 2 Chromatogram of []

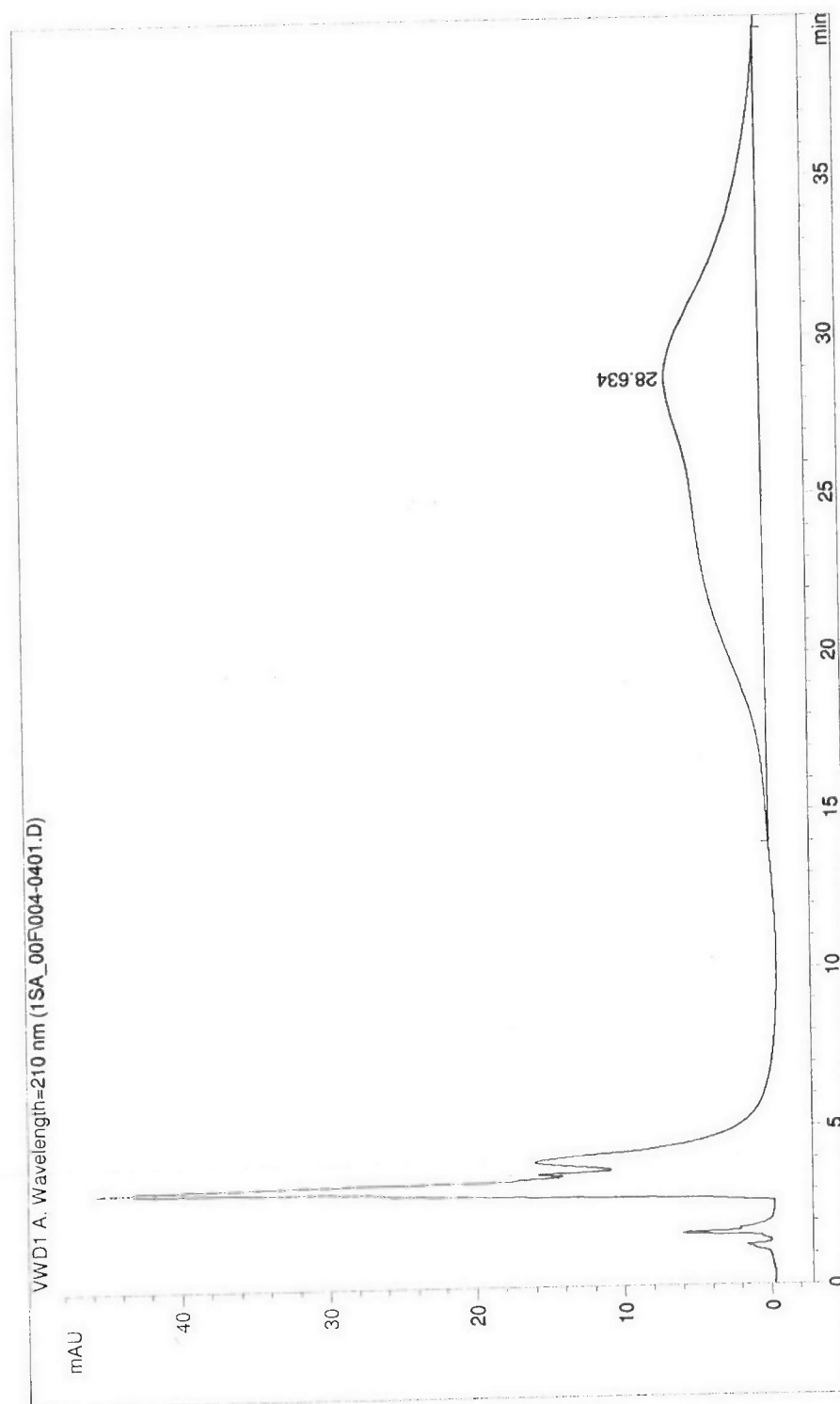
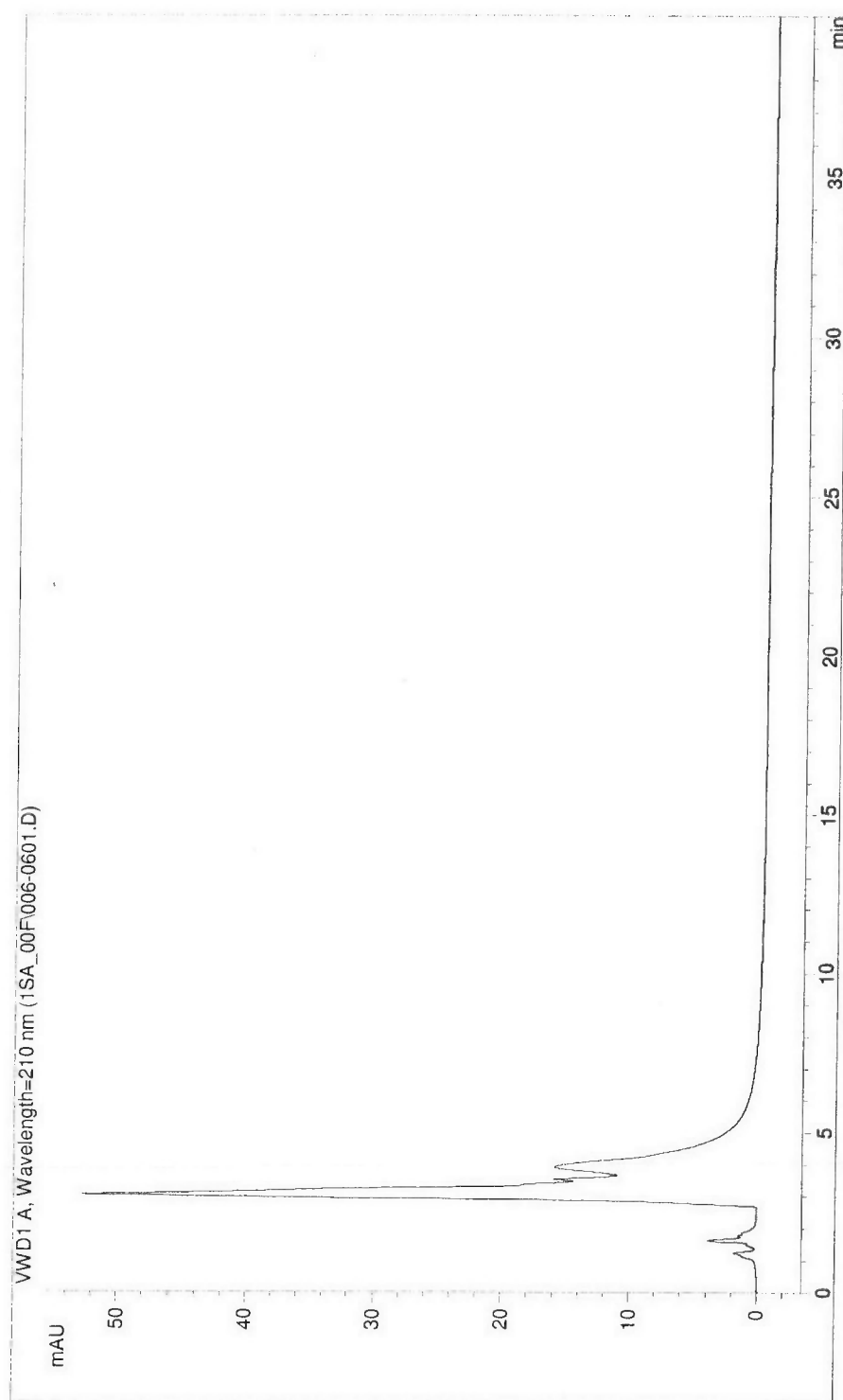


Figure 3 Chromatogram of solvent blank (methanol:tetrahydrofuran (9:1 v/v))





**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Huntingdon Life Sciences
Eye Research Centre
Occold
Eye
Suffolk
IP23 7PX

TEST TYPE

Analytical/Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

17-19 February 2009

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

A handwritten signature in black ink, appearing to be 'A. Gray', with the date '1/5/09' written below it.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

The logo for the Medicines and Healthcare products Regulatory Agency (MHRA), consisting of the letters 'MHRA' in white inside a black oval.

ALGAL GROWTH INHIBITION TEST

PROJECT NUMBER: 2737/0003

AUTHOR: H Vryenhoef

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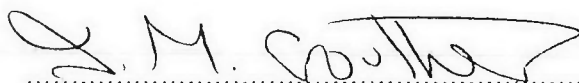
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

13 November 2008	Standard Test Method Compliance Audit
05 May 2009	Test Material Preparation
11 May 2009	Test System Preparation
12 May 2009	Exposure
22 May 2009	Assessment of Response
15, 18 May 2009	Chemical Analysis
§ 18 June 2009	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§ Evaluation specific to this study	



For the Quality Assurance Unit*

DATE: 26 JUN 2009

***Authorised QA Signatures:**

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

.....  DATE: 25 JUN 2009
H Vryenhoef BSc
Study Director

This report may be presented in final form as a digital (pdf) document. Such documents are prepared by scanning the paper original, and are considered of equivalent integrity and authenticity to versions produced by optical photocopy. However, in all cases the hand-signed paper original, held in secure archives, is the definitive document.

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ALGAL GROWTH INHIBITION TEST

SUMMARY

Introduction. A study was performed to assess the effect of the test material on the growth of the green alga *Desmodesmus subspicatus*. The method followed that described in the OECD Guidelines for Testing of Chemicals (2006) No 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" referenced as Method C.3 of Commission Regulation (EC) No 440/2008.

Methods. Information provided by the Sponsor indicated that the test material was insoluble in water. Pre-study solubility work conducted indicated that it was not possible to obtain a testable solution of the test material using traditional methods of preparation e.g. ultrasonication and high shear mixing.

A pre-study media preparation trial indicated that a dissolved test material concentration of approximately 0.0041 mg/l was obtained from a saturated solution method of preparation indicating this to be the limit of water solubility of this material under test conditions.

Following a preliminary range-finding test, *Desmodesmus subspicatus* was exposed to solutions of the test material at 0-Hour measured concentrations of 0.00035, 0.00082, 0.0021, 0.0037 and 0.014 mg/l (three replicate flasks per concentration) for 72 hours, under constant illumination and shaking at a temperature of $24 \pm 1^{\circ}\text{C}$. The test material solutions were prepared by stirring an excess (50 mg/l) of test material in culture medium using a propeller stirrer at approximately 1500 rpm at a temperature of approximately 21°C for 24 hours. After the stirring period any undissolved test material was removed by centrifugation at 10000 g for 30 minutes to produce a saturated solution of the test material with a 0-Hour measured test concentration of 0.014 mg/l. This saturated solution was then further diluted as necessary, to provide the remaining test groups.

The difference observed between the measured concentration obtained in the pre-study media preparation trial and definitive test was considered to be due to the different diluent types used.

Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Coulter® Multisizer Particle Counter.

Results. Exposure of *Desmodesmus subspicatus* to the test material based on the 0-Hour measured test concentrations gave EC_{50} values of greater than 0.014 mg/l and correspondingly the No Observed Effect Concentration was 0.014 mg/l.

Analysis of the test preparations at 0 hours showed measured test concentrations to range from 0.00035 to 0.014 mg/l. A decline in measured test concentrations was observed at 72 hours in the range of less than the limit of quantitation (LOQ) of the analytical method employed which was determined to be 0.00017 mg/l to 0.0026 mg/l. This decline was in line with the preliminary stability analyses conducted which indicated that the test material was unstable in culture medium.

Given this decline in measured test concentrations it was considered justifiable to base the results on the geometric mean measured test concentrations in order to give a "worst case" analysis of the data.

The EC_{50} values based on the geometric mean measured test concentrations were greater than 0.0060 mg/l and correspondingly the No Observed Effect Concentration was 0.0060 mg/l.

This study showed that there were no toxic effects at saturation.

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ALGAL GROWTH INHIBITION TEST

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the effect of the test material on the growth of the green alga *Desmodesmus subspicatus*. The method followed the recommendations of the OECD Guidelines for Testing of Chemicals (2006) No 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" referenced as Method C.3 of Commission Regulation (EC) No 440/2008.


Desmodesmus subspicatus (formerly known as *Scenedesmus subspicatus*) is a freshwater unicellular alga, representative of primary producers found in natural waters and can therefore be considered as an important non-target organism in freshwater ecosystems.

The study was conducted between 30 April 2009 and 09 June 2009.

In view of the difficulties associated with the evaluation of aquatic toxicity of poorly water soluble test materials, a modification of the standard method for the preparation of aqueous media was performed. An approach endorsed by several important regulatory authorities in the EU and elsewhere (ECETOC 1996 and OECD 2000), is to expose organisms to a saturated solution of the test material in cases where the test material is of high purity and is poorly soluble in water and in the permitted auxiliary solvents and surfactants. Using this approach, a saturated solution was prepared by stirring an excess (50 mg/l) of test material in culture medium for a period of 24 hours prior to removing any undissolved test material present by centrifugation at 10000 *g* for 30 minutes to give a saturated solution of the test material.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification : 

Description : white powder

Batch number : 20090105

Date received : 23 January 2009

Storage conditions : room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor. A Certificate of Analysis for the test material supplied by the Sponsor is given in Appendix 1.

3. METHODS

3.1 Test Species

The test was carried out using *Desmodesmus subspicatus* strain CCAP 276/20. Liquid cultures of *Desmodesmus subspicatus* were obtained from the Culture Collection of Algae and Protozoa (CCAP), Dunstaffnage Marine Laboratory, Oban, Argyll, Scotland. Master cultures were maintained in the laboratory by the periodic replenishment of culture medium (Section 3.2). The master cultures were maintained in the laboratory under constant aeration and constant illumination at $21 \pm 1^\circ\text{C}$.

Prior to the start of the test sufficient master culture was added to approximately 100 ml volumes of culture media contained in conical flasks to give an initial cell density of approximately 10^3 cells/ml. The flasks were plugged with polyurethane foam stoppers and kept under constant agitation by orbital shaker (100 – 150 rpm) and constant illumination at $24 \pm 1^\circ\text{C}$ until the algal cell density was approximately 10^4 - 10^5 cells/ml.

A positive control (Harlan Laboratories Ltd Project Number: 0039/1066) used potassium dichromate as the reference material. Details of the positive control are given in Appendix 2. The positive control was conducted between 18 November 2008 and 21 November 2008

3.2 Culture Medium

The culture medium used for both the range-finding and definitive tests was the same as that used to maintain the stock culture.

The culture medium is defined in Appendix 3.

3.3 Procedure

3.3.1 Pre-study media preparation trial

Information provided by the Sponsor indicated that the test material was insoluble in water. Pre-study solubility work conducted indicated that it was not possible to obtain a testable solution of the test material using traditional methods of preparation e.g. ultrasonication and high shear mixing.

Preliminary solubility work conducted indicated that the test material was practically insoluble in water using traditional methods of preparation e.g. ultrasonication and high shear mixing.

Based on this information the test material was categorised as being a 'difficult substance' as defined by the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 2000). Therefore a media preparation trial was conducted in order to determine the solubility of the test material under test conditions.

An amount of test material (550 mg) was dispersed, in duplicate, in 11 litres of reconstituted water with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 21°C for periods of 24 or 48 hours. After stirring samples were taken for chemical analysis after the following pre-treatments:

- Centrifugation at 10000 g for 30 minutes
- Centrifugation at 40000 g for 30 minutes
- Filtration through a 0.2 µm Sartorius Sartopore filter (approximately 500 ml discarded in order to pre-condition the filter)
- Filtration through a 0.2 µm Sartorius Sartopore filter (approximately 1 litre discarded in order to pre-condition the filter)

3.3.2 Range-finding test

The results obtained from the pre-study media preparation trial conducted indicated that a dissolved test material concentration of approximately 0.0041 mg/l could be obtained using a saturated solution method of preparation.

The test concentrations to be used in the definitive test were determined by a preliminary range-finding test. The range-finding test was conducted by exposing *Desmodesmus subspicatus* cells to a series of nominal test concentrations of 0.000041, 0.00041 and 0.0041 mg/l for a period of 72 hours.

An amount of test material (550 mg) was dispersed in 11 litres of culture medium with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 21°C for 24 hours. After 24 hours the stirring was stopped and any undissolved test material was removed by centrifugation at 10000 g for 30 minutes to give a saturated solution with a nominal concentration of 0.0041 mg/l. A series of dilutions was made from this saturated solution to give further stock solutions of 0.00041 and 0.000041 mg/l. An aliquot (250 ml) of each of the stock solutions was separately inoculated with algal suspension (1.1 ml) to give the required test concentrations of 0.000041, 0.00041 and 0.0041 mg/l.

The test was conducted in 250 ml glass conical flasks each containing 100 ml of test preparation and plugged with polyurethane foam bungs to reduce evaporation. Two replicate flasks were used for each control and test concentration.

The control group was maintained under identical conditions but not exposed to the test material.

At the start of the range-finding test a sample of each test and control culture was removed and the cell density determined using a Coulter® Multisizer Particle Counter. The flasks were then plugged with polyurethane foam bungs and incubated (INFORS Multitron® Version 2 incubator) at $24 \pm 1^\circ\text{C}$ under continuous illumination (intensity approximately 7000 lux) provided by warm white lighting (380 – 730 nm) and constantly shaken at approximately 150 rpm for 72 hours.

After 72 hours the cell density of each flask was determined using a Coulter® Multisizer Particle Counter.

3.3.3 Definitive test

Based on the results of the pre-study media preparation trial and range-finding test the following nominal test concentrations were assigned to the definitive test: 0.000041, 0.00013, 0.00041, 0.0013 and 0.0041 mg/l.

Chemical analysis of the test preparations at 0 hours showed measured test concentrations of 0.00035, 0.00082, 0.0021, 0.0037 and 0.014 mg/l. The differences observed between the measured test concentrations obtained for the pre-study media preparation trial and definitive test were considered to be due to the different diluent types used.

3.3.3.1 *Experimental Preparation*

Due to the low aqueous solubility and high purity of the test material the test concentrations used in the definitive test were prepared by diluting (with culture medium) a saturated solution prepared from an initial test material dispersion at a concentration of 50 mg/l.

An amount of test material (550 mg) was dispersed in 11 litres of culture medium with the aid of propeller stirring at approximately 1500 rpm at a temperature of approximately 21°C for 24 hours. After 24 hours the stirring was stopped and any undissolved test material was removed by centrifugation at 10000 g to give a saturated solution with a 0-Hour measured test concentration of 0.014 mg/l. A series of dilutions was made from this saturated solution to give further stock solutions of 0.0037, 0.0021, 0.00082 and 0.00035 mg/l. An aliquot (900 ml) of each of the stock solutions was separately inoculated with 3.9 ml algal suspension to give the test concentrations of 0.00035, 0.00082, 0.0021, 0.0037 and 0.014 mg/l.

The stock solutions and each of the prepared concentrations were inverted several times to ensure adequate mixing and homogeneity.

The concentration and stability of the test material in the test solutions were verified by chemical analysis at 0 and 72 hours (see Appendix 4).

3.3.3.2 Exposure conditions

As in the range-finding test 250 ml glass conical flasks were used. Six flasks each containing 100 ml of solution were used for the control and three flasks each containing 100 ml were used for each treatment group.

The control group was maintained under identical conditions but not exposed to the test material.

Pre-culture conditions gave an algal suspension in log phase growth characterised by a cell density of 9.29×10^5 cells per ml. Inoculation of 900 ml of test medium with 3.9 ml of this algal suspension gave an initial nominal cell density of 4×10^3 cells per ml and had no significant dilution effect on the final test concentration.

The flasks were plugged with polyurethane foam bungs and incubated (INFORS Multitron® Version 2 incubator) at $24 \pm 1^\circ\text{C}$ under continuous illumination (intensity approximately 7000 lux) provided by warm white lighting (380 – 730 nm) and constantly shaken at approximately 150 rpm for 72 hours.

Samples were taken at 0, 24, 48 and 72 hours and the cell densities determined using a Coulter® Multisizer Particle Counter.

3.3.3.3 Physico-chemical measurements

The pH of each control and test flask was determined at initiation of the test and after 72 hours exposure. The pH was measured using a WTW pH 320 pH meter. The temperature within the incubator was recorded daily.

3.3.3.4 Verification of test concentrations

Samples were taken from the control (replicates $R_1 - R_6$ pooled) and each test group (replicates $R_1 - R_3$ pooled) at 0 and 72 hours for quantitative analysis. Duplicate samples were taken at 0 hours and stored at approximately -20°C for further analysis if necessary. Sample volumes required for chemical analysis precluded the storage of duplicate samples at 72 hours.

The method of analysis, stability, recovery and test solution analyses are described in Appendix 4.

3.3.4 Evaluation of data

3.3.4.1 Comparison of growth rates

The average specific growth rate for a specified period is calculated as the logarithmic increase in biomass from the equation:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

where:

- μ = average specific growth rate from time t_1 to t_n
- N_1 = cell concentration at t_1
- N_n = cell concentration at t_n
- t_1 = time of first measurement
- t_n = time of n^{th} measurement

The average specific growth rate over the test duration was calculated for each replicate control and test material vessel using the nominally inoculated cell concentration as the starting value rather than the measured starting value in order to increase the precision of the calculation.

In addition the section by section specific growth rate (days 0-1, 1-2 and 2-3) was calculated for the control cultures and the results examined in order to determine whether the growth rate remained constant.

Percentage inhibition of growth rate for each replicate test material vessel was calculated using the following equation:

$$I_r = \frac{\mu_c - \mu_t}{\mu_c} \times 100$$

where:

- I_r = percentage inhibition of average specific growth rate
 μ_c = mean average specific growth rate for the control cultures
 μ_t = average specific growth rate for the test culture

3.3.4.2 Comparison of Yield

Yield is calculated as the increase in biomass over the exposure period using the following equation:

$$Y = N_n - N_0$$

where:

- Y = yield
 N_0 = cell concentration at the start of the test
 N_n = cell concentration at the end of the test

For each test concentration and control the mean value for yield along with the standard deviation was calculated. Percentage inhibition of yield was calculated using the following equation:

$$I_y = \frac{(Y_c - Y_t)}{Y_c} \times 100$$

where:

- I_y = percentage inhibition of yield
 Y_c = mean value for yield in the control group
 Y_t = mean value for yield for the treatment group

3.3.4.3 Determination of EC_x values

For each individual test vessel (mean values for yield), percentage inhibition (arithmetic axis) was plotted against test concentration (logarithmic axis) and a line fitted by computerised interpolation using the Xlfit software package (IDBS). EC_x values were then determined from the equation for the fitted line.

3.3.4.4 Statistical analysis

One way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett 1955) was carried out on the growth rate and yield data after 72 hours for the control and all test concentrations to determine any statistically significant differences between the test and control groups. All statistical analyses were performed using the SAS computer software package (SAS 1999 - 2001).

3.3.4.5 Geometric mean measured test concentrations

The geometric mean measured test concentrations of the samples were calculated as follows using the measured test concentrations of replicates R_1 - R_3 pooled:

$$GM = \sqrt{C_0 \times C_1}$$

where

- GM = geometric mean measured test concentration (mg/l)
- C_0 = measured concentration at the start of the test (mg/l)
- C_1 = measured concentration at the end of the test (mg/l)

3.4 Validation Criteria

The results of the test are considered valid if the following performance criteria are met:

- The cell concentration of the control cultures must increase by a factor of at least 16 over the test period.
- The mean of the coefficients of variation of the section by section daily growth rates in the control cultures during the course of the test (days 0-1, 1-2 and 2-3, for 72-Hour tests) must not exceed 35%.
- The coefficient of variation of the average specific growth rate in replicate control cultures must not exceed 7%.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Pre-Study Media Preparation Trial

The results obtained from the pre-study media preparation trial conducted (see Appendix 4) indicated that the test material was adsorbing to the filter matrices. There was no significant increase in the dissolved test material concentration obtained when the preparation period of the saturated solution was extended to 48 hours.

Based on this information the test material was prepared using a saturated solution method of preparation at an initial loading rate of 50 mg/l, stirred for a period of 24 hours prior to removal of any undissolved test material by centrifugation at 10,000 g for 30 minutes to give a nominal test concentration of approximately 0.0041 mg/l.

5.2 Range-finding Test

The cell densities and percentage inhibition of growth values from the exposure of *Desmodesmus subspicatus* to the test material during the range-finding test are given in Table 1.

The results showed no effect on growth at the nominal test concentration of 0.000041 mg/l. However, growth was observed to be reduced at 0.00041 and 0.0041 mg/l.

Based on this information nominal test concentrations of 0.000041, 0.00013, 0.00041, 0.0013, and 0.0041 mg/l were selected for the definitive test.

Chemical analysis of the test preparations at 0 hours showed measured test concentrations of 0.00035, 0.00082, 0.0021, 0.00037 and 0.014 mg/l. The differences observed between the measured test concentrations obtained for the pre-study media preparation trial and definitive test were considered to be due to the different diluent types used.

5.3 Definitive Test

Cell density values determined at each sampling time and pH values at 0 and 72 hours are given in Table 2. Daily specific growth rates for the control cultures are given in

Table 3. Growth rate and yield values for the control and test cultures after 72 hours and percentage inhibition values are given in Table 4.

The mean cell densities versus time for the definitive test are presented in Figure 1.

5.3.1 Validation criteria

The following data show that the cell concentration of the control cultures increased by a factor of 48 after 72 hours. This increase was in line with the OECD Guideline that states the enhancement must be at least by a factor of 16 after 72 hours.

Mean cell density of control at 0 hours : 4.09×10^3 cells per ml

Mean cell density of control at 72 hours : 1.96×10^5 cells per ml

The mean coefficient of variation for section by section specific growth rate for the control cultures was 35% and hence satisfied the validation criterion given in the OECD Guideline which states the mean must not exceed 35%.

The coefficient of variation for average specific growth rate for the control cultures over the test period (0 – 72 h) was 4% and hence satisfied the validation criterion given in the OECD Guideline which states that this must not exceed 7%.

5.3.2 Growth data

From the data given in Tables 2 and 4, it is clear that the growth rate (r), yield (y) and biomass (b) of *Desmodesmus subspicatus* (CCAP 276/20) were not affected by the presence of the test material over the 72-Hour exposure period. Based on these results it is considered that the inhibition observed during the range-finding test was due to possible contamination and not a true toxic response.

Accordingly the following results were determined from the data based on the 0-Hour measured test concentrations:

5.3.2.1 Inhibition of growth rate

E_rC_{10} (0 - 72 h) : > 0.014 mg/l

E_rC_{20} (0 - 72 h) : > 0.014 mg/l

E_rC_{50} (0 - 72 h) : > 0.014 mg/l

where E_rC_x is the test concentration that reduced growth rate by x%.

Statistical analysis of the growth rate data was carried out for the control and all test concentrations using one way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett 1955). There were no statistically significant decreases in growth rate between the control, 0.00035, 0.00082, 0.0021, 0.0037 and 0.014 mg/l test concentrations ($P \geq 0.05$) and therefore the "No Observed Effect Concentration" (NOEC) based on growth rate was 0.014 mg/l.

5.3.2.2 Inhibition of yield

E_yC_{10} (0 - 72 h) : > 0.014 mg/l
 E_yC_{20} (0 - 72 h) : > 0.014 mg/l
 E_yC_{50} (0 - 72 h) : > 0.014 mg/l

where E_yC_x is the test concentration that reduced yield by x%.

Statistical analysis of the yield data was carried out as in Section 5.3.2.1. There were no statistically significant differences ($P \geq 0.05$), between the control, 0.00035, 0.00082, 0.0021, 0.0037 and 0.014 mg/l test groups and therefore the "No Observed Effect Concentration" (NOEC) based on yield was 0.014 mg/l.

5.3.3 Observations on cultures

All test and control cultures were inspected microscopically at 72 hours. There were no abnormalities detected in any of the control or test cultures.

5.3.4 Observations on test material solubility

At the start of the test all control and test cultures were observed to be clear colourless solutions. After the 72-Hour test period all control and test cultures were observed to be pale green dispersions.

5.3.5 Physico-chemical measurements

The pH values of each test and control flask are given in Table 2. Temperature was maintained at $24 \pm 1^\circ\text{C}$ throughout the test.

The pH values of the control cultures (see Table 2) were observed to increase from pH 7.3 – 7.7 at 0 hours to pH 7.6 – 7.9 at 72 hours. The pH deviation in the control cultures was less than 1.5 pH units after 72 hours and therefore was within the limits given in the Test Guidelines.

5.3.6 Verification of test concentrations

Chemical analysis of the test preparations at 0 hours (see Appendix 4) showed measured test concentrations to range from 0.00035 to 0.014 mg/l. A decline in measured test concentrations was observed at 72 hours in the range of less than the limit of quantitation (LOQ) of the analytical method employed which was determined to be 0.00017 mg/l to 0.0026 mg/l.

This decline was in line with the preliminary stability analyses conducted which indicated that the test material was unstable in culture medium. Current regulatory advice is that in cases where a decline in measured concentrations is observed, geometric mean measured concentrations should be used for calculating EC₅₀ values. It was therefore considered justifiable to base the results on the geometric mean measured test concentrations in order to give a "worst case" analysis of the data. In cases where the measured concentration was less than the LOQ of the analytical method following current regulatory advice a value of half the LOQ (i.e. 0.000085 mg/l) was used to enable calculation of the geometric mean measured concentration. The geometric mean measured test concentrations were determined to be:

0-Hour Measured Test Concentration (mg/l)	Geometric Mean Measured Test Concentration (mg/l)	Expressed as a % of the 0-Hour Measured Test Concentration
0.00035	0.00017	48
0.00082	0.00026	32
0.0021	0.00084	40
0.0037	0.0018	49
0.014	0.0060	44

The following results were determined from the data based on the geometric mean measured test concentrations:

Growth rate

E_rC_{10} (0 - 72 h) : > 0.0060 mg/l

E_rC_{20} (0 - 72 h) : > 0.0060 mg/l

E_rC_{50} (0 - 72 h) : > 0.0060 mg/l

No Observed Effect Concentration (NOEC) = 0.0060 mg/l

Yield

E_yC_{10} (0 - 72 h) : > 0.0060 mg/l

E_yC_{20} (0 - 72 h) : > 0.0060 mg/l

E_yC_{50} (0 - 72 h) : > 0.0060 mg/l

No Observed Effect Concentration (NOEC) = 0.0060 mg/l

The use of the geometric mean measured test concentrations in the calculation of the EC_{50} and NOEC values had no significant effect on the outcome of the study.

6. CONCLUSION

The effect of the test material on the growth of *Desmodesmus subspicatus* has been investigated and based on the 0-Hour measured test concentrations gave EC_{50} values of greater than 0.014 mg/l. Correspondingly the No Observed Effect Concentration was 0.014 mg/l.

Based on the geometric mean measured test concentrations exposure of *Desmodesmus subspicatus* to the test material gave EC_{50} values of greater than 0.0060 mg/l. Correspondingly the No Observed Effect Concentration was 0.0060 mg/l.

This study showed that there were no toxic effects at saturation.

7. REFERENCES

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ALGAL GROWTH INHIBITION TEST

Table 1 **Cell Densities and Percentage Inhibition of Growth from the Range-finding Test**

Nominal Concentration (mg/l)		Cell Densities* (cells per ml)		Inhibition Values (%)	
		0 Hours	72 Hours	Growth Rate	Yield
Control	R ₁	4.19E+03	2.01E+05	-	-
	R ₂	4.18E+03	2.43E+05		
	Mean	4.19E+03	2.22E+05		
0.000041	R ₁	4.11E+03	3.85E+05	[13]	[56]
	R ₂	4.04E+03	3.03E+05		
	Mean	4.08E+03	3.44E+05		
0.00041	R ₁	4.10E+03	1.01E+05	15	45
	R ₂	4.06E+03	1.47E+05		
	Mean	4.08E+03	1.24E+05		
0.0041	R ₁	4.02E+03	1.67E+05	11	40
	R ₂	4.14E+03	1.02E+05		
	Mean	4.08E+03	1.34E+05		

* Cell densities represent the mean number of cells per ml calculated from the mean of the cell counts from 3 counts for each of the replicate flasks.

R₁ and R₂ = Replicates 1 and 2

[Increase in growth compared to controls]

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ALGAL GROWTH INHIBITION TEST

Table 2 Cell Densities and pH Values in the Definitive Test

0-Hour Measured Test Concentration (mg/l)		pH	Cell Densities* (cells per ml)					pH
		0 h	0 h	24 h	48 h	72 h	72 h	
Control	R ₁	7.7	4.01E+03	9.04E+03	3.63E+04	2.30E+05	7.9	
	R ₂	7.5	4.11E+03	9.13E+03	4.10E+04	2.13E+05	7.9	
	R ₃	7.5	4.01E+03	9.98E+03	3.37E+04	1.87E+05	7.8	
	R ₄	7.4	4.15E+03	1.10E+04	3.17E+04	1.74E+05	7.7	
	R ₅	7.4	4.09E+03	7.98E+03	3.56E+04	2.19E+05	7.7	
	R ₆	7.3	4.15E+03	9.02E+03	4.20E+04	1.53E+05	7.6	
	Mean		4.09E+03	9.36E+03	3.67E+04	1.96E+05		
0.00035	R ₁	7.3	4.04E+03	1.36E+04	3.56E+04	2.54E+05	7.6	
	R ₂	7.3	4.07E+03	1.12E+04	4.07E+04	1.97E+05	7.7	
	R ₃	7.3	4.05E+03	8.65E+03	4.19E+04	1.93E+05	7.6	
	Mean		4.05E+03	1.11E+04	3.94E+04	2.15E+05		
0.00082	R ₁	7.3	4.02E+03	1.34E+04	3.44E+04	2.45E+05	7.7	
	R ₂	7.3	4.02E+03	1.18E+04	2.58E+04	2.21E+05	7.7	
	R ₃	7.3	4.07E+03	9.66E+03	2.72E+04	2.36E+05	7.6	
	Mean		4.04E+03	1.16E+04	2.92E+04	2.34E+05		
0.0021	R ₁	7.3	4.08E+03	9.27E+03	2.76E+04	1.86E+05	7.6	
	R ₂	7.3	4.04E+03	9.76E+03	3.31E+04	2.78E+05	7.6	
	R ₃	7.3	4.02E+03	7.85E+03	1.81E+04	1.77E+05	7.6	
	Mean		4.05E+03	8.96E+03	2.63E+04	2.13E+05		
0.0037	R ₁	7.3	4.08E+03	9.03E+03	2.75E+04	2.66E+05	7.6	
	R ₂	7.3	4.06E+03	6.98E+03	2.49E+04	2.55E+05	7.6	
	R ₃	7.3	4.09E+03	9.78E+03	3.00E+04	3.00E+05	7.6	
	Mean		4.08E+03	8.60E+03	2.75E+04	2.73E+05		
0.014	R ₁	7.2	4.02E+03	1.12E+04	3.40E+04	2.20E+05	7.6	
	R ₂	7.3	4.10E+03	1.25E+04	3.65E+04	3.25E+05	7.6	
	R ₃	7.3	4.00E+03	1.35E+04	4.54E+04	2.60E+05	7.6	
	Mean		4.04E+03	1.24E+04	3.86E+04	2.68E+05		

* Cell densities represent the mean number of cells per ml calculated from the mean of the cell counts from 3 counts for each of the replicate flasks.

R₁ - R₆ = Replicates 1 to 6

ALGAL GROWTH INHIBITION TEST

Table 3 **Daily Specific Growth Rates for the Control Cultures in the Definitive Test**

		Daily Specific Growth Rate (cells/ml/hour)		
		Day 0 - 1	Day 1 - 2	Day 2 - 3
Control	R ₁	0.034	0.058	0.077
	R ₂	0.034	0.063	0.069
	R ₃	0.038	0.051	0.071
	R ₄	0.042	0.044	0.071
	R ₅	0.029	0.062	0.076
	R ₆	0.034	0.064	0.054
	Mean	0.035	0.057	0.070

R₁ - R₆ = Replicates 1 to 6

ALGAL GROWTH INHIBITION TEST

Table 4 Inhibition of Growth Rate and Yield in the Definitive Test

0-Hour Measured Test Concentration (mg/l)		Growth Rate (cells/ml/hour)		Yield (cells/ml)	
		0 – 72 h	% Inhibition	0 – 72 h	% Inhibition*
Control	R ₁	0.056	-	2.26E+05	-
	R ₂	0.055		2.09E+05	
	R ₃	0.053		1.83E+05	
	R ₄	0.052		1.70E+05	
	R ₅	0.056		2.15E+05	
	R ₆	0.051		1.49E+05	
	Mean	0.054		1.92E+05	
	SD	0.002		2.97E+04	
0.00035	R ₁	0.058	[7]	2.50E+05	[10]
	R ₂	0.054	0	1.93E+05	
	R ₃	0.054	0	1.89E+05	
	Mean	0.055	[2]	2.11E+05	
	SD	0.002		3.40E+04	
0.00082	R ₁	0.057	[6]	2.41E+05	[20]
	R ₂	0.056	[4]	2.17E+05	
	R ₃	0.057	[6]	2.32E+05	
	Mean	0.057	[5]	2.30E+05	
	SD	0.001		1.19E+04	
0.0021	R ₁	0.053	2	1.81E+05	[9]
	R ₂	0.059	[9]	2.74E+05	
	R ₃	0.053	2	1.73E+05	
	Mean	0.055	[2]	2.09E+05	
	SD	0.003		5.61E+04	
0.0037	R ₁	0.058	[7]	2.62E+05	[40]
	R ₂	0.058	[7]	2.51E+05	
	R ₃	0.060	[11]	2.96E+05	
	Mean	0.059	[8]	2.69E+05	
	SD	0.001		2.34E+04	
0.014	R ₁	0.056	[4]	2.16E+05	[38]
	R ₂	0.061	[13]	3.21E+05	
	R ₃	0.058	[7]	2.56E+05	
	Mean	0.058	[8]	2.64E+05	
	SD	0.003		5.29E+04	

* In accordance with the OECD test guideline only the mean value for yield for each test concentration is calculated

R₁ – R₆ = Replicates 1 to 6

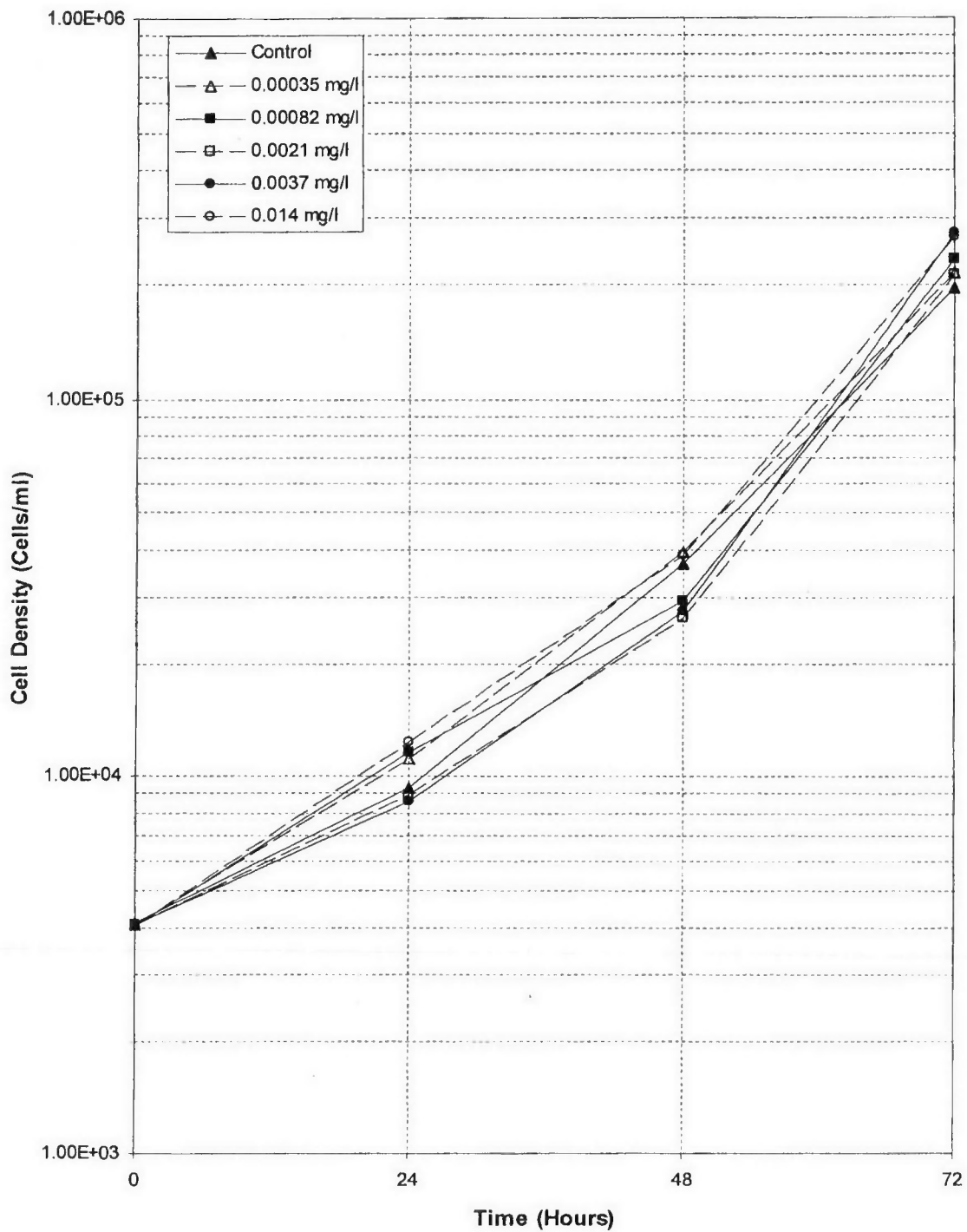
SD = Standard Deviation

[Increase in growth as compared to controls]

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ALGAL GROWTH INHIBITION TEST

Figure 1 Mean Cell Densities v Time for the Definitive Test



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ALGAL GROWTH INHIBITION TEST

Appendix 1 Certificate of Analysis

Certificate of Analysis

1. TEST MATERIAL IDENTIFICATION

- 1) PRODUCT NAME : []
- 2) TEST MATERIAL(LOT No.) : 20090105
- 3) QUANTITY : 100g
- 4) CHEMICAL NAME : []
- 5) COMPOSITIONS OF CHEMICAL

- 6) PURITY : >99%
- 7) APPEARANCE : White Powder
- 8) MOLECULAR WEIGHT : []
- 9) MOLECULAR FORMULA : []

2. SOLUBILITY :

- 1) INSOLUBLE : H₂O
- 2) SOLUBLE : Toluene(Partially)

3. STORAGE :

- 1) Storage temperature : Room Temperature
- 2) Expiry date : Jan. 08. 2011

We hereby certify that the data stated here above are true and correct

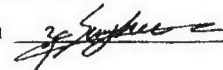
Company(Manufacturer) : CHEIL INDUSTRIAL INC.

Address : (437-711) 332-2, Gocheon-dong, Uiwang-si, Gyeonggi-do, Korea

Date : Jan. 09. 2009

Name of person in charge

Sunghee Ahn



ALGAL GROWTH INHIBITION TEST

Appendix 2 Positive Control

A positive control (Harlan Laboratories Ltd Project No: 0039/1066) used potassium dichromate as the reference material at concentrations of 0.0625, 0.125, 0.25, 0.50 and 1.0 mg/l.

Exposure conditions and data evaluation for the positive control were similar to those in the definitive test.

Exposure of *Desmodesmus subspicatus* (CCAP 276/20) to the reference material gave the following results:

E_rC_{50} (0 – 72 h)	:	0.52 mg/l, 95% confidence limits 0.43 – 0.62 mg/l
E_yC_{50} (0 – 72 h)	:	0.29 mg/l, 95% confidence limits 0.25 – 0.33 mg/l

No Observed Effect Concentration (NOEC) based on growth rate:	0.125 mg/l
No Observed Effect Concentration (NOEC) based on yield:	0.125 mg/l
Lowest Observed Effect Concentration (LOEC) based on growth rate:	0.25 mg/l
Lowest Observed Effect Concentration (LOEC) based on yield:	0.25 mg/l

The results from the positive control with potassium dichromate were within the normal ranges for this reference material.

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ALGAL GROWTH INHIBITION TEST

Appendix 3 Culture Medium

NaNO ₃	25.5	mg/l
MgCl ₂ .6H ₂ O	12.164	mg/l
CaCl ₂ .2H ₂ O	4.41	mg/l
MgSO ₄ .7H ₂ O	14.7	mg/l
K ₂ HPO ₄	1.044	mg/l
NaHCO ₃	15.0	mg/l
H ₃ BO ₃	0.1855	mg/l
MnCl ₂ .4H ₂ O	0.415	mg/l
ZnCl ₂	0.00327	mg/l
FeCl ₃ .6H ₂ O	0.159	mg/l
CoCl ₂ .6H ₂ O	0.00143	mg/l
Na ₂ MoO ₄ .2H ₂ O	0.00726	mg/l
CuCl ₂ .2H ₂ O	0.000012	mg/l
Na ₂ EDTA.2H ₂ O	0.30	mg/l
Na ₂ SeO ₃ .5H ₂ O	0.000010	mg/l

The culture medium was prepared using reverse osmosis purified deionised water* and the pH adjusted to 7.5 ± 0.1 with 0.1N NaOH or HCl.

* Elga Optima 15+ or Elga Purelab Option R-15 BP

ALGAL GROWTH INHIBITION TEST**Appendix 4 Verification of Test Concentrations****1. METHOD OF ANALYSIS****1.1 Introduction**

The test material concentration in the test samples was determined by high performance liquid chromatography (HPLC) using an external standard. The test material gave a chromatographic profile consisting of a number of peaks. The results have been calculated using the main peak associated with the test material.

The method was developed by the Department of Analytical Services, Harlan Laboratories Ltd, Shardlow, UK.

1.2 Sample Preparation

A C8 (EC) solid phase extraction (SPE) cartridge (500 mg/3 ml) was packed with glass wool prior to sequentially pre-conditioning with methanol and water*. A volume of test sample was eluted through the cartridge and the cartridge dried. The test material was eluted from the cartridge with methanol and made to volume to give a final theoretical concentration of approximately 0.0062 to 0.62 mg/l.

1.3 Standards

Standard solutions of test material were prepared initially in tetrahydrofuran at a nominal concentration of 1000 mg/l, standards diluted to give a final theoretical concentration of 0.50 mg/l in methanol containing 10% tetrahydrofuran.

* Prepared by ELGA Purelab Option R-15 water purification

ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations

1.4 Procedure

The standards and samples were analysed by HPLC using the following conditions:

HPLC System	:	Agilent Technologies 1050 or 1100, incorporating autosampler and workstation
Column	:	Symmetry, C18, 3.5 μ , (50 x 3.0 mm id)
Column temperature	:	40°C
Mobile phase	:	methanol
Flow rate	:	0.8 ml/min
UV/Vis detector wavelength	:	210 nm
Injection volume	:	50 μ l
Retention time	:	approximately 1.4 minutes

2. PRE-STUDY MEDIA PREPARATION TRIAL

An amount of test material (550 mg) was dispersed, in duplicate, in 11 litres of reconstituted water. These were stirred using a propeller stirrer at approximately 1500 rpm at approximately 21°C for periods of 24 and 48 hours.

Samples were taken for analysis following removal of any undissolved test material by centrifugation at 10000 or 40000 g for 30 minutes or following filtration through 0.2 μ m Sartorius Sartopore filters with the first approximate 500 or 1000 ml being discarded.

Stirring Period and Treatment	Concentration Found (mg/l)
24 Hours Control	<LOQ
24 Hours Centrifuged 10000 g	0.00410
24 Hours Centrifuged 40000 g	0.00261
24 Hours Filtered 500 ml discarded	<LOQ
24 Hours Filtered 1000 ml discarded	<LOQ

LOQ = Limit of quantitation

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ALGAL GROWTH INHIBITION TEST**Appendix 4 (continued) Verification of Test Concentrations**

Stirring Period and Treatment	Concentration Found (mg/l)
48 Hours Control	<LOQ
48 Hours Centrifuged 10000 g	0.00539
48 Hours Centrifuged 40000 g	0.00624
48 Hours Filtered 500 ml discarded	<LOQ
48 Hours Filtered 1000 ml discarded	<LOQ

The above results have been corrected for a recovery rate of 79%.

3. VALIDATION**3.1 Linearity**

A range of standard solutions covering 0.025 to 0.74 mg/l (exceeding the range of the working sample concentrations) was analysed.

Linearity was confirmed ($R^2 = 0.9981$) in the range 0 to 0.74 mg/l.

The results are presented graphically on page 34.

3.2 Recoveries

Preliminary test samples, accurately fortified at known concentrations of test material, were prepared and analysed.

The recovery samples were prepared by addition of a standard solution of test material to a sample of test medium. A standard solution was accurately prepared by initially dissolving the test material in tetrahydrofuran which was further diluted in methanol. An accurate volume of the standard solution was added to a known volume of test medium to achieve the required concentration of test material.

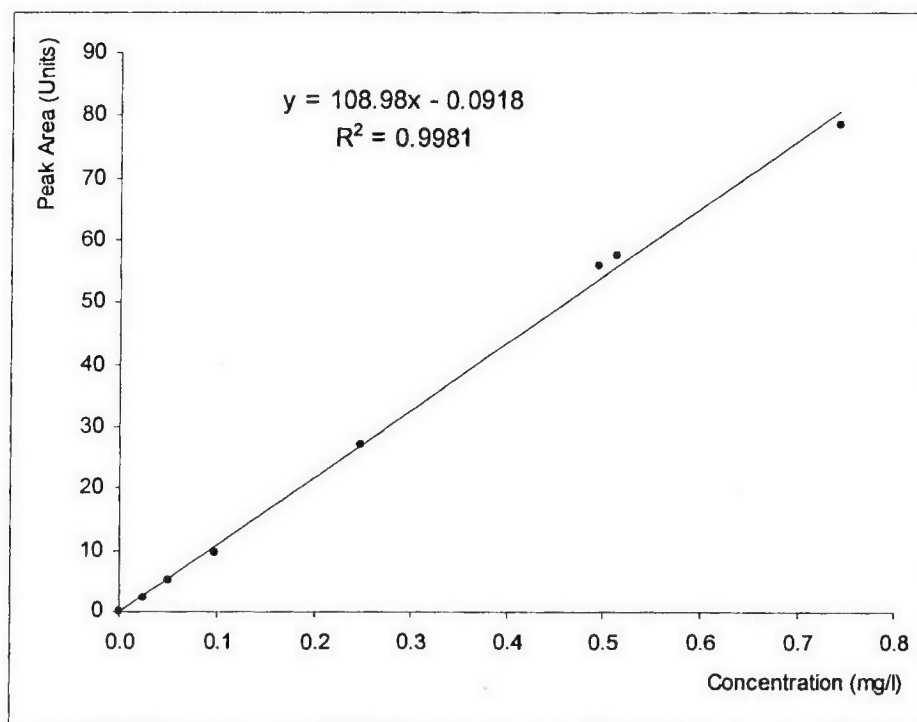
Further portions of test samples were analysed following the addition of algal cells to assess the effects of algae on the recovery of test material from test medium.

LOQ = Limit of quantitation

ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations

Linearity of Detector Response



ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations

Fortification (mg/l)	Recoveries		
	(mg/l)	(%)	Mean %
0.000401	0.000239	60	63
0.000401	0.000265	66	
0.00401	0.00179	45	49
0.00401	0.00217	54	
0.00401 plus algae	0.00241	60	-

The recovery results are below the acceptance limits of 80 - 120% therefore all test sample results will be corrected for an overall mean recovery rate of 56.094%

The method has been considered to be sufficiently accurate for the purposes of this test.

The presence of algal cells was considered to have no significant effect on the recovery of the test material from the medium.

The limit of quantitation has been assessed down to 0.00017 mg/l.

4. STABILITY

Preliminary test samples were prepared, analysed initially and then after storage in sealed glass vessels at ambient temperature in light and dark conditions for approximately 72 hours (equivalent to the test exposure period). In addition a test sample was tested for stability without prior mixing (sonication) of the test sample bottle to assess for losses due to adsorption and/or insolubility.

Nominal concentration (mg/l)	0.00041	0.0041
Concentration found initially (mg/l)	0.000252	0.00198
Concentration found after storage in light conditions (mg/l)	<LOQ	0.00149
Expressed as a percent of the initial concentration	-	75
Concentration found after storage in dark conditions (mg/l)	<LOQ	0.00106
Expressed as a percent of the initial concentration	-	54
Concentration found after storage in dark conditions (mg/l) - unsonicated sample	<LOQ	0.00126
Expressed as a percent of the initial concentration	-	63

LOQ = Limit of quantitation

ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations

The test samples have been shown to be unstable in the test medium in light and dark conditions.

The unsonicated stability vessel showed no evidence of insolubility or adherence to glass.

5. RESULTS

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)*
0 hours	Control	<LOQ
	0.000041	0.000351
	0.00013	0.000818
	0.00041	0.00212
	0.0013	0.00365
	0.0041	0.0135
72 hours	Control	<LOQ
	0.000041	<LOQ
	0.00013	<LOQ
	0.00041	0.000330
	0.0013	0.000843
	0.0041	0.00264

6. DISCUSSION

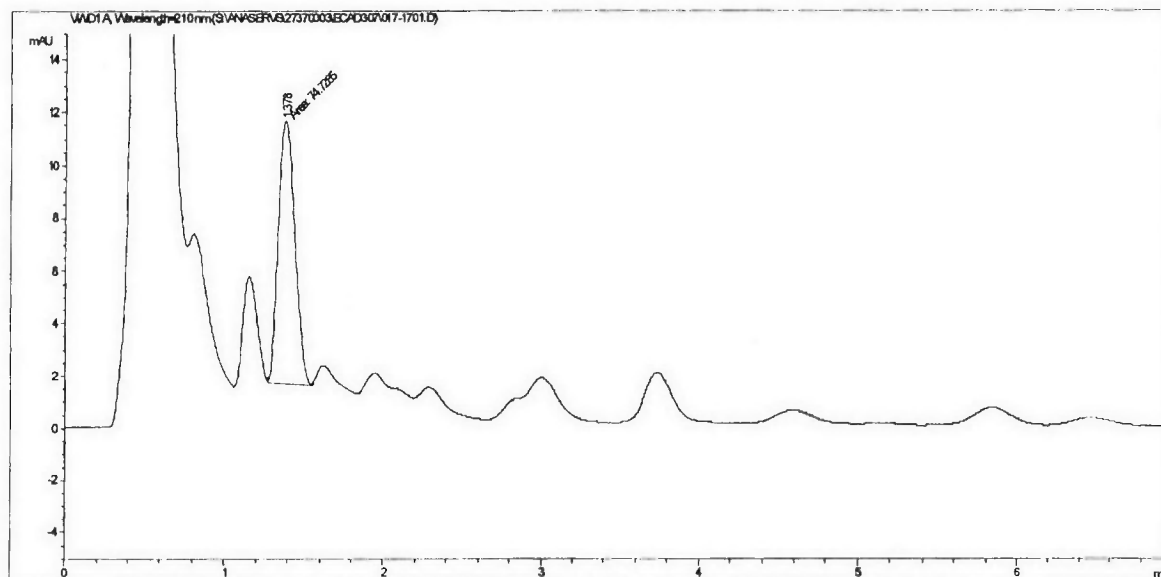
The detection system was found to have acceptable linearity. The analytical procedure had low recoveries of test material in test medium which was overcome by correction of an overall recovery rate of 56.094%. A method of analysis was validated and proven to be suitable for use.

* Results corrected for an overall mean recovery of 56.094%
LOQ = Limit of quantitation

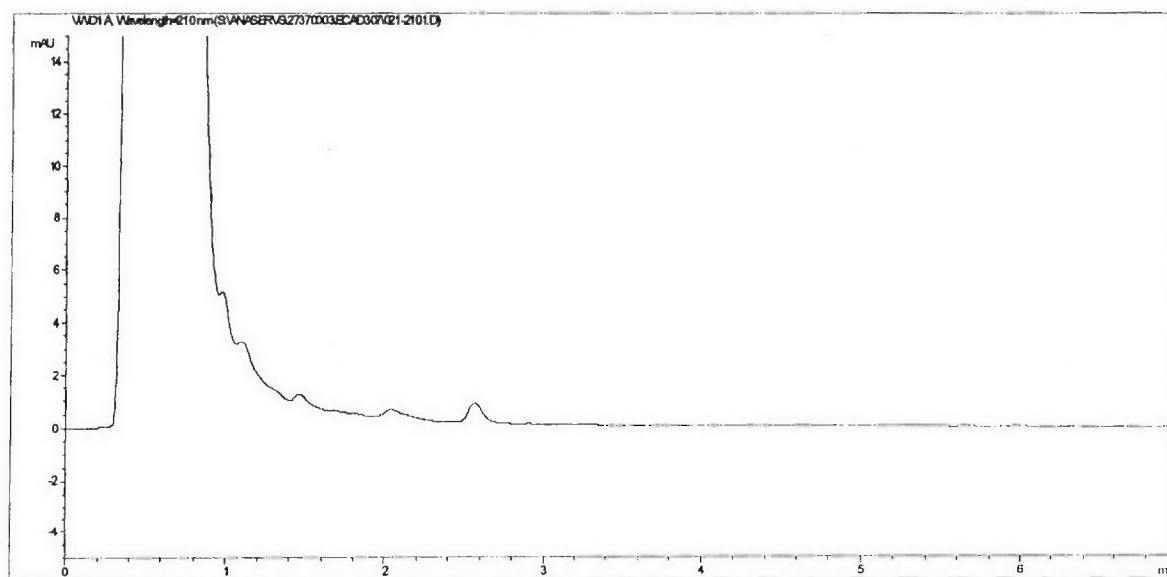
ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations

7. TYPICAL CHROMATOGRAPHY



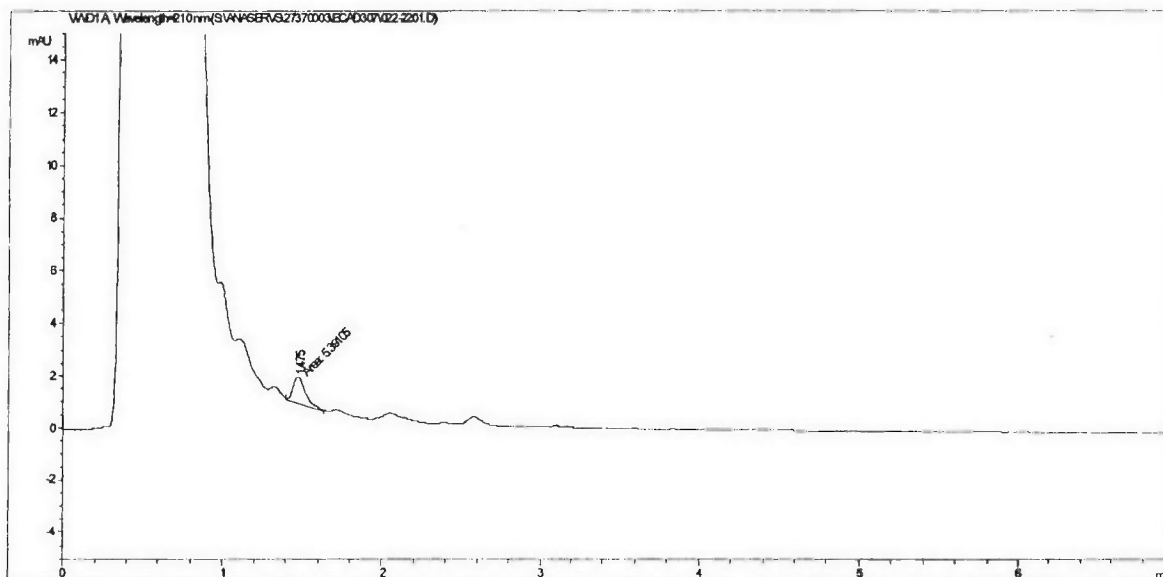
Standard 0.50 mg/l



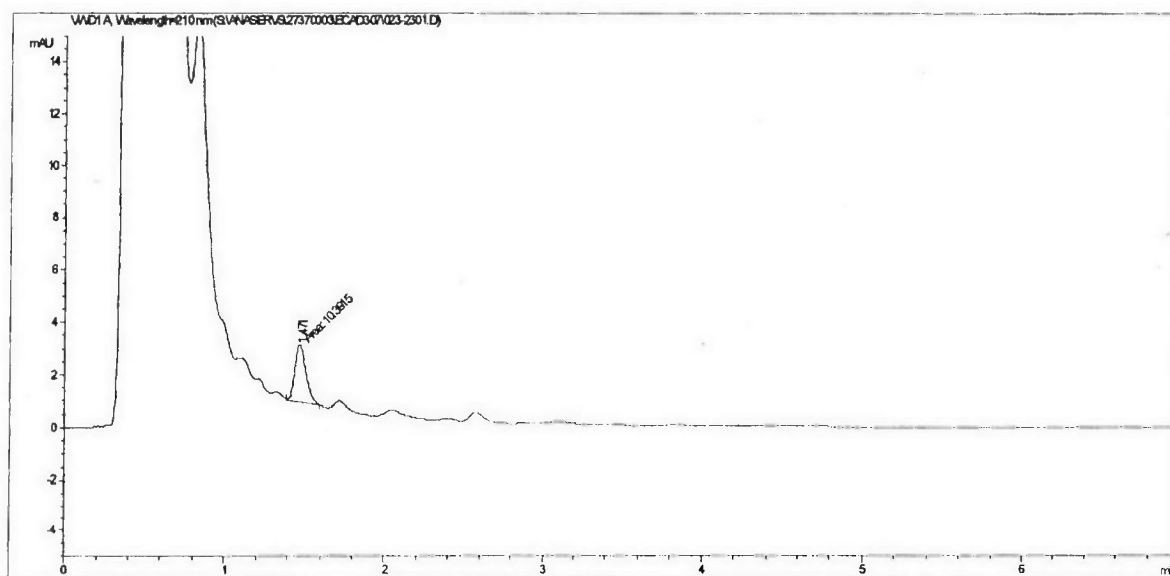
Control Sample

ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations



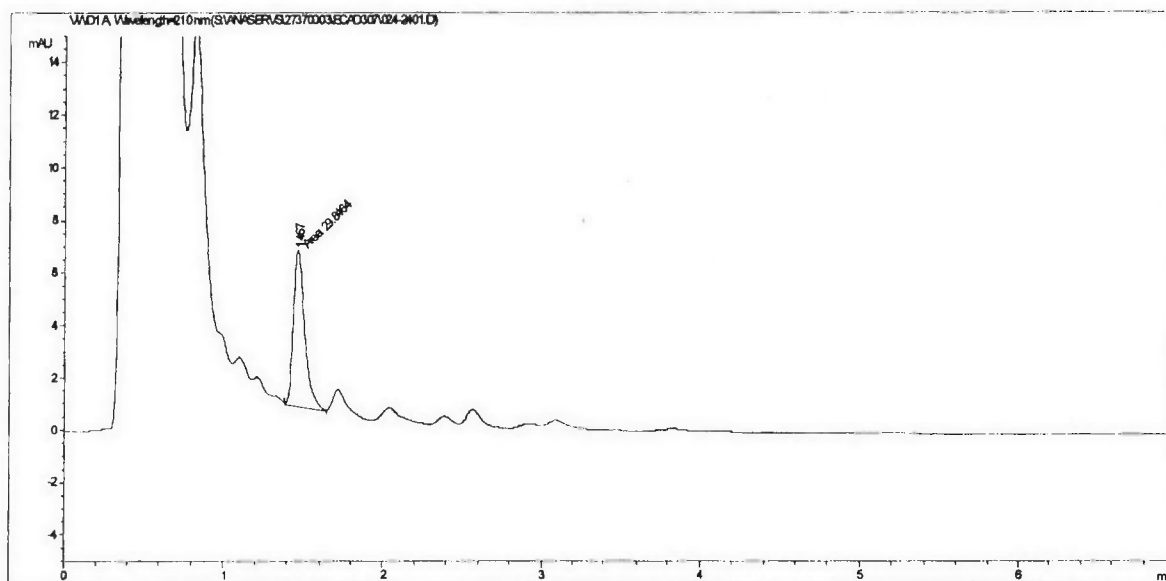
Test Sample 0.000041 mg/l



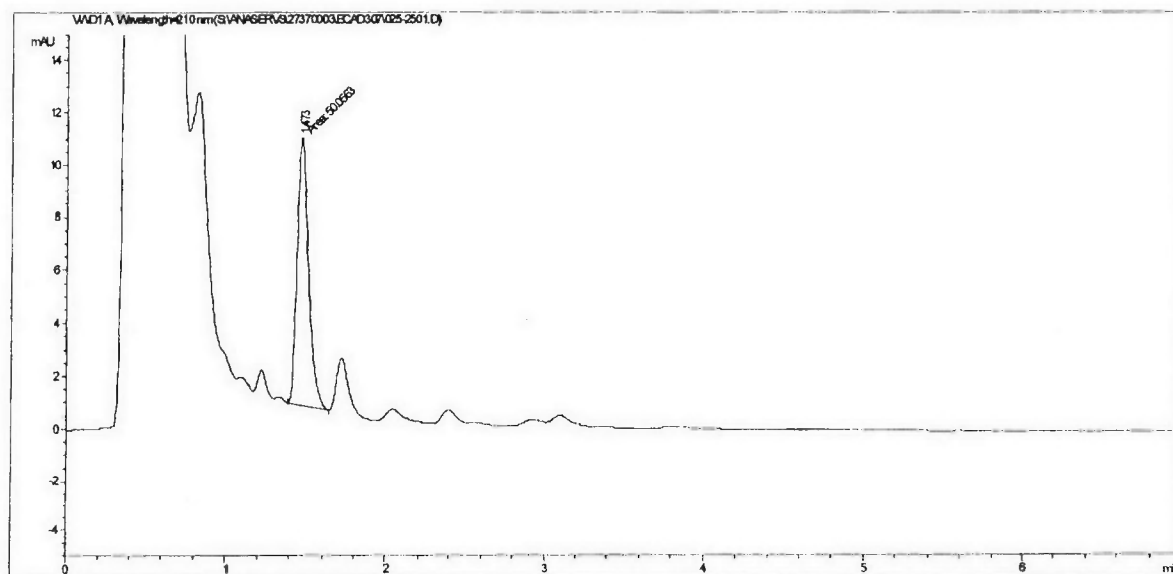
Test Sample 0.00013 mg/l

ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations



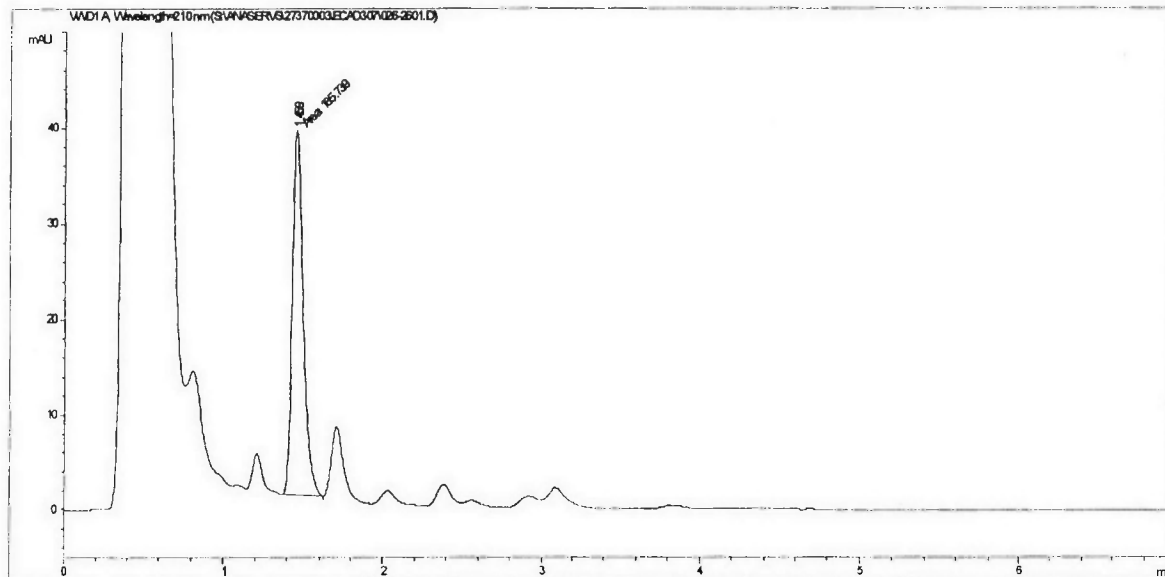
Test Sample 0.00041 mg/l



Test Sample 0.0013 mg/l

ALGAL GROWTH INHIBITION TEST

Appendix 4 (continued) Verification of Test Concentrations



Test Sample 0.0041 mg/l

**Appendix 5 Statement of GLP Compliance in Accordance with Directive
2004/9/EC**



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Harlan Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicology
Mutagenicity
Phys/Chem
Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


4/3/09
Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

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Focus Report
New Chemicals Program
PMN Number: **P-09-0631**

Focus Date: 10/04/2009 11:00:00 PM Report Status: Completed
Consolidated Set:
Focus Chair: Darlene Jones Contractor: Christina Stanley

I. Notice Information

Submitter: [REDACTED] CAS Number: [REDACTED]
Chemical Name: [REDACTED]
Use: Flame retardant for molded electrical devices. No references were found for the PMN material. All analogs are flame retardants.
Other Uses: [REDACTED]
PV-Max: [REDACTED] Kg/yr
Manufacture: Import: X

II. SAT Results

(1) **Health Rating:** 2 **Eco Rating:** 3 **Comments:** ;
Occupational: 2-3A **Non-Occupational:** 2 **Environmental:** 1
(1) **PBT:** 3 2 2 **Comments:**

III. OTHER FACTORS

Categories:

Health Chemical Category: Ecotox Category: [REDACTED]

Related Cases/Regulatory History:

Health related Cases:
Ecotox Related Cases: Analogs: [REDACTED]
Regulatory History:

MSDS/Label Information:

MSDS: Yes Label: No
General Equipment: rubber gloves/ chemical safety glasses with side shields or chemical safety goggles/ local exhaust ventilation/ general mechanical ventilation
Respirator: [REDACTED]
Health Effects: hazards of this material have not been fully investigated/ not expected to be acutely toxic/ exposure to skin, eyes, and inhalation not expected to be acutely toxic/ mechanical irritation is possible to the eye and to the mucous membranes if inhaled
TLV/PEL (PMN or raw material): [REDACTED]

[REDACTED] No
Exposure Based (Environmental):

IV. Summary of SAT Assessment

Fate:

Fate Summary: P-09-0631
FATE: Estimations for typical [REDACTED] material MW [REDACTED]
[REDACTED]

PBT Potential: P3B2

*CEB FATE: [REDACTED]

Health:

Health Summary:

Not absorbed through the skin as the neat material (pchem), poor absorption through the skin if in solution (analog), poor absorption from the lung and GI tract (pchem). Concern for mutagenicity [REDACTED] both (+) in a mouse micronucleus assay]; eye irritation based on submitted test data; dermal sensitization based on reports on workers in 8E- [REDACTED] and 8E- [REDACTED] for [REDACTED]; heritable mutagenicity and uncertain concern for male reproductive toxicity based on effects at 5000 and 10,000 mg/kg in an ip dominant lethal assay with [REDACTED]. Also concern for "slight" effects to the pituitary, pancreas, adrenals, blood, kidney, liver, and thyroid in various 28 - 106 day studies with Pyro-chek 77B [lowest LOAEL = 100 ppm (13.7 mg/kg) in a 28-day feeding study in rats]; and oncogenicity based on [REDACTED], some evidence in male and female rats, equivocal evidence in male mice, and no evidence in female mice].

Test Data:

(-) Salmonella with and without activation; (-) E. coli with and without activation; (-) for chromosome aberrations in V79 cells with and without activation; rat oral LD0 = 2000 mg/kg; no skin irritation in rabbits; mild eye irritation in rabbits; (-) for skin sensitization in a mouse local lymph node assay up to 25% ai; rat 28-day oral NOAEL = 1000 mg/kg (highest dose tested)

Ecotox:

Ecotox Values:

Fish 96-h LC50:	*(P)	0.0034(M)
Daphnid 48-h LC50:	*(P)	0.0011(M)
Green algal 96-h EC50:	*(P)	0.006(M)
Fish Chronic Value:	*(P)	
Daphnid ChV:	*(P)	
Algal ChV:	*(P)	

Ecotox values comments: Predictions are based on SARs for neutral organic chemicals; SAR chemical class = aryl halide; MW 656 (most abundant congener); log Kow = 10.1 (EPI); pH7; effective concentrations based on 100% active ingredients and nominal concentrations; hardness <180.0 mg/L as CaCO3; and TOC <2.0 mg/L;

This PMN came with ecotoxicity data. Attached spreadsheet shows details. Below are conclusions:

All three tests are considered valid. The OECD Difficult to Test Substance guidance document was consulted in the preparation of the test material and it was decided that it was not possible to obtain a testable solution of the PMN material using traditional methods of preparation due to the insolubility of the test compound. The test solution was prepared in tetrahydrofuran and analyzed centrifuged and non-centrifuged to determine the total amount of test material present. Chemical analysis of the centrifuged test preparations showed measured concentrations lower than the 0.004 mg/L nominal target concentration. This was considered to be due to the differences in media or sampling techniques used to remove the supernatant after centrifugation. Therefore, concentrations are considered as time-weighted mean measured or geometric means depending on the toxicity test. The species observed to be most sensitive to exposure of the PMN substance is daphnia therefore the concern concentration is derived from the 48-hr acute LC50 for daphnia of 0.0011

mg/L. The concern concentration is determined by dividing this value by an acute-to-chronic ratio of 10 to yield 0.00011 mg/L which is then divided again by an assessment (uncertainty factor) of ten to yield 0.011 µg/L. This value is rounded to the nearest whole value to yield 1 µg/L or 1 ppb. The PMN P09-0631 is of high concern for aquatic toxicity.

Ecotox Factors:

Assessment Factor: 10

Concern Concentration: 1

V. [REDACTED]

[illegible][illegible]

VI. Focus Decision and Rationale

Regulatory Actions

Regulatory Decision: PMN Ban Pending Upfront Testing

Decision Date: 10/04/2009

Type of Decision:

Rationale:

P0P-09-631 will be regulated under the TSCA section 5(e) categories for neutral organics (ecotoxicity and environmental fate) and PBT (health, ecotoxicity, and environmental fate). The PMN will also be regulated under the [REDACTED] for human health concerns (PBT). Human health concerns were moderate due to concerns for mutagenicity, eye irritation, dermal sensitization, heritable mutagenicity, uncertain concern for male reproductive systems, "slight" effects to the pituitary, pancreas, adrenals, blood, kidney, liver, and thyroid, and oncogenicity. The Inhalation Monitoring Criteria for the Pilot Program were met for [REDACTED]. [REDACTED] e EAB persistent exposure based criteria were also met for inhalation dose: [REDACTED] mg/kg/d. Ecotoxicity concerns were high and risks are from releases to water where the 1.00 ppb COC was exceeded [REDACTED] days/yr (SWC: [REDACTED]). The PBT score for this submission is P3B2T2. Testing to address the risk-based concerns for PBT will follow the 5(e) category requirements. The chronic fish study should be performed on the rainbow trout. If the PMN substance proves not to be a PBT then testing should be performed to address the ecotoxicity and environmental fate concerns under the category for neutral organics. If the PMN substance is shown to be a PBT and all of the testing under the PBT category is complete, then the company must perform the OECD 308 test to address the concerns for the neutral organics category. To address the concerns for ecotoxicity and environmental fate under the 5(e) category for neutral organics the tests will include the daphnid chronic toxicity test (OPPTS 850.1300), fish early-life stage toxicity test (OPPTS 850.1400) with rainbow trout, and an aerobic and anaerobic transformation in aquatic sediment systems (OECD 308). To address the exposure-based human health concerns the testing will be the Mammalian Erythrocyte Micronucleus Test (Intraperitoneal route) (OPPTS 870.5395) and the combined repeated dose toxicity study with reproduction/developmental toxicity screening test (OECD 422) modified by extending the exposure period to 90-days. Depending on the results of the OECD 422 a combined toxicity/carcinogenicity study may be required.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]
SWC: 7.22 ppb

DW: LADD: 4.02E-06 mg/kg/d, ADR: 3.52E-04 mg/kg/d

Fish: LADD: 7.10E-06 mg/kg/d, ADR: 1.05E-03 mg/kg/d

>COC (1.00 ppb) [REDACTED] d/yr

[REDACTED]
LADD: 1.48E-05 mg/kg/d, ADR: 5.07E-04 mg/kg/d

[REDACTED]
LADD: 1.64E-04 mg/kg/d, ADR: 1.41E-02 mg/kg/d

P2 Rec Comments:

Testing:

Final Recommended:

Health: mouse micro-nucleus combined repeated dose study extended to [REDACTED]
days of exposure (OECD 422)
Eco: OECD 308
Fate:
Other:

SAT Report

PMN Number: **P-09-0631**

SAT Date: **9/25/2009**

Print Date: **2/24/2015**

Related cases:

Health related cases:

Ecotox related cases: Analog: [REDACTED]

Concern levels:

Type of Concern:	<u>Health</u>	<u>Eco</u>	<u>Comments</u>
Level of Concern:	2	3	

<u>Persistence</u>	<u>Bioaccum</u>	<u>Toxicity</u>	<u>Comments</u>
3	2	2	
		Awaiting	
		Human Health	
		Entry	
		Awaiting	
		Human Health	
		Entry	
		Awaiting	
		Human Health	
		Entry	

Exposure Based Review:

Health:

Ecotox: No

Routes of exposure:

Health: Dermal Drinking Water Inhalation

Ecotox: All releases to water

Fate: ;

Keywords:

Keywords:

Summary of Assessment:

Fate:

Fate Summary: P-09-0631

FATE: Estimations for typical [REDACTED] material MW [REDACTED]

[REDACTED]

[REDACTED]



Health:

Health Summary: Not absorbed through the skin as the neat material (pchem), poor absorption through the skin if in solution (analog), poor absorption from the lung and GI tract (pchem). Concern for mutagenicity [redacted] both (+) in a mouse micronucleus assay]; eye irritation based on submitted test data; dermal sensitization based on reports on workers in [redacted] and [redacted] for [redacted] [redacted] heritable mutagenicity and uncertain concern for male reproductive toxicity based on effects at 5000 and 10,000 mg/kg in an ip dominant lethal assay with [redacted] Also concern for "slight" effects to the pituitary, pancreas, adrenals, blood, kidney, liver, and thyroid in various 28 - 106 day studies with [redacted] 77B [lowest LOAEL = 100 ppm (13.7 mg/kg) in a 28-day feeding study in rats]; and oncogenicity based on [redacted], some evidence in male and female rats, equivocal evidence in male mice, and no evidence in female mice].

Test Data: (-) Salmonella with and without activation; (-) E. coli with and without activation; (-) for chromosome aberrations in V79 cells with and without activation; rat oral LD0 = 2000 mg/kg; no skin irritation in rabbits; mild eye irritation in rabbits; (-) for skin sensitization in a mouse local lymph node assay up to 25% ai; rat 28-day oral NOAEL = 1000 mg/kg (highest dose tested)

Ecotox:

Test Organism	Test Type	Test End Point	Predicted	Measured	Comments
fish	96-h	LC50	*	0.0034	
daphnid	48-h	LC50	*	0.0011	
green algal	96-h	EC50	*	0.006	
fish	—	chronic value	*		
daphnid	—	chronic value	*		

algal	–	chronic value	*		
Sewage Sludge	3-h	EC50	–		
Sewage Sludge	–	Chronic Value	–		

Ecotox Values Comments:

Factors	Values	Comments
Assessment Factor	10	
Concentration of Concern (ppb)	1	Results do not agree with ECOSAR predictions based on the [REDACTED] of the substance and because of the use of the [REDACTED]. However, because this substance is a [REDACTED] are inherent toxicity concerns for aquatic organisms. The measured data, despite the test material being prepared via the use of a disperant, will determine toxicity.
SARs	[REDACTED]	
SAR Class	[REDACTED]	
Ecotox Category		

Ecotox Factors Comments:

SAT Chair: L Keifer 564-8916

INITIAL REVIEW ENGINEERING REPORT

P-09-0631

Focus Ready Draft 10/4/2009 11:00:00 PM

ENGINEER: Avcin \ DDH

PV (kg/yr):

Revision Notes/Assessment Overview:

SUBMITTER: . (submitter)

USE: Flame retardant for molded electrical devices. No references were found for the PMN material. All analogs are flame retardants.

MSDS: Yes

LABEL: No

Gen Eqpt: rubber gloves/ chemical safety glasses with side shields or chemical safety goggles/ local exhaust ventilation/ general mechanical ventilation

Respirator

Health Effects: hazards of this material have not been fully investigated/ not expected to be acutely toxic/ exposure to skin, eyes, and inhalation not expected to be acutely toxic/ mechanical irritation is possible to the eye and to the mucous membranes if inhaled

TLV/PEL: - ,

LVE PPE:

CRSS: (9/23/2009 11:00:00 PM):

Chemical Name:

S-H2O:

VP:

MW:

Submitted and estimated physical properties are on page 6 of this report.

Consumer Use:

SAT (concerns): (9/24/2009 11:00:00 PM):

Migration to groundwater:

PBT rating: P3 B2 T2

Health: 2, Dermal, Drinking Water, Inhalation

Eco: 1, No releases to Water

OCCUPATIONAL EXPOSURE RATING: 2-3A

NOTES & KEY ASSUMPTIONS:

Generated by the 06/07/2005 version of ChemSTEER. The submitter was called; see contact report. The PMN is import only, therefore, manufacturing was not assessed. /// The PMN is used as a flame retardant for molded electrical devices.

///

[REDACTED]

This IRER assessed dermal exposures per SAT requirements.

POLLUTION PREVENTION CONSIDERATIONS:

None.

P2 REC:

EXPOSURE-BASED REVIEW: [REDACTED]

P-09-0631

Proc/Use: Flame Retardant in Plastic Products

Number of Sites/Location: submitter site(s)

unknown site

unknown site

Basis: Submission estimates █ sites, █ d/yr, imported in █ or █ kg bags. CEB assumes █ kg bags.

Process Description: Imported PMN ([REDACTED] per CRSS), submission [REDACTED]

ENVIRONMENTAL RELEASES ESTIMATE SUMMARY

IRER

Output 2: [redacted] kg/site-day over [redacted] day/yr from [redacted] sites or [redacted] kg/yr

to: Landfill (technical contact)

from:

Output 2: [REDACTED] kg/site-day over [REDACTED] day/yr from [REDACTED] sites or [REDACTED] kg/yr

to:

Output _____ kg/site-day over _____ day/yr from _____ sites or _____ kg/yr

to:

RELEASE TOTAL

kg/yr -

OCCUPATIONAL EXPOSURES ESTIMATE SUMMARY

Tot. # of workers exposed via assessed routes:

Basis:

Inhalation:

Exposure to Particulate

Upper Bound: [REDACTED] mg/day over [REDACTED] days/yr

Number of workers (all sites) with [REDACTED] :

Basis: [REDACTED] .

INHALATION MONITORING DATA REVIEW

1) Uncertainty (estimate based on model, regulatory limit, or data not specific to industry): [REDACTED]

2) (a) Exposure level > 1 mg/day? [REDACTED]

(b) Hazard Rating for health of 2 or greater? [REDACTED]

Inhalation Monitoring Data Desired? [REDACTED])

Dermal:

[REDACTED]
High End: [REDACTED] mg/day over [REDACTED] days/yr


Number of workers (all sites) with Dermal exposure: [REDACTED]

Basis: [REDACTED] [REDACTED]

[REDACTED]

Chemical ID: P090631

Results Table: Dose, Concentration, and Days Exceeded Results Summary

Exposure Scenario ¹	Water						Landfill	Stack Air		Fugitive Air	
Release activity(ies) ² ; exposure calculation(s) ³	Drinking Water		Fish Ingestion		7Q10 ⁴ CC=1	PDM Days Exceeded	LADD	ADR	LADD	ADR	LADD
	ADR	LADD	ADR	LADD							
	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	µg/l	# Days	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
Proc/Use: max ADR, max acute eco, PDM, max LADD	3.52E-04	4.02E-06	1.05E-03	7.10E-06	7.22		---	5.07E-04	1.48E-05	1.41E-02	1.64E-04

² Release activities are from engineering report's Manufacturing (Mfg), Processing (Proc) and Use release activity labels.

³ Exposure calculations are Acute Dose Rate (ADR), Lifetime Average Daily Dose (LADD), and Probabilistic Dilution Model (PDM). There may be one, two, or all three exposure calculations per exposure scenario.

CC is the aquatic concentration of concern.

⁴ This column displays concentration values for the 7Q10 streamflow, which is defined as the average streamflow of the 7 consecutive days of lowest flow within a 10 year period.

Remarks:

Results Table: Exposure Based (XB)/Persistent (P2B2) Criteria

[illegible]

Fate test recommendations: OECD 308 (aerobic and anaerobic transformation in water/sediment)=OPPTS 835.4300 and 835.4400

INITIAL REVIEW

Chemical ID: P090631

Assessor: Delpire

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Scenario#:1

Number of

Activity: Proc/Use: max ADR, max acute eco, PDM, max LADD

Description:				

Total :				
	(kg/yr)	(kg/yr)	(kg/yr)	(kg/yr)

	(kg/site/day)	(kg/site/day)	(kg/site/day)	(kg/site/day)

Remarks:

INITIAL REVIEW EXPOSURE REPORT

Chemical ID: P090631

SIC-CODE BASED HUMAN AND AQUATIC EXPOSURES TO

max acute eco, PDM, max LADD

SIC-CODE DESCRIPTION: POTW (Indust., includes POTWs which .)

SIC-CODE (S):

EXPOSED POPULATION: Adult

					kg)

PLANT TYPE	% ILE FACILITY	STREAM FLOW (MLD)				STREAM CONC. (µg/l)			

DRINKING WATER AND FISH INGESTION EXPOSURE ESTIMATES						
Exposure Units	Drinking Water Results		Drinking Water Units	Fish Ingestion Results		Fish Ingestion Units
	50%	10%		50%	10%	
Cancer						
LADD _{pot}	5.53E-07	4.02E-06	mg/kg/day	9.76E-07	7.10E-06	mg/kg/day
LADC _{pot}	2.83E-05	2.06E-04	mg/L	1.17E-02	8.49E-02	mg/kg
Acute						
ADR _{pot}	3.78E-05	3.52E-04	mg/kg/day	1.44E-04	1.05E-03	mg/kg/day

SIC Code Comments:

INITIAL REVIEW EXPOSURE REPORT

Chemical ID: P090631

SIC CODE EXPOSURES TO SURFACE

SCENARIO #: 1

RELEASE ACTIVITY: Proc/Use: max ADR, max acute eco, PDM, max LADD

SIC CODE DESCRIPTION:

ASSOCIATED SIC CODES:

SIC CODE RESULTS

COC (µg/L)						
1.00						

Remark: COC is exceeded greater than

INITIAL [REDACTED]
 Chemical ID: P090631

[REDACTED] ESTIMATES (POST-TREATMENT)

SCENARIO #: 1

RELEASE ACTIVITY: Proc/Use: max ADR, max acute eco, PDM, max LADD

RELEASE DESCRIPTION:

METHOD OF CALCULATION: Screen3

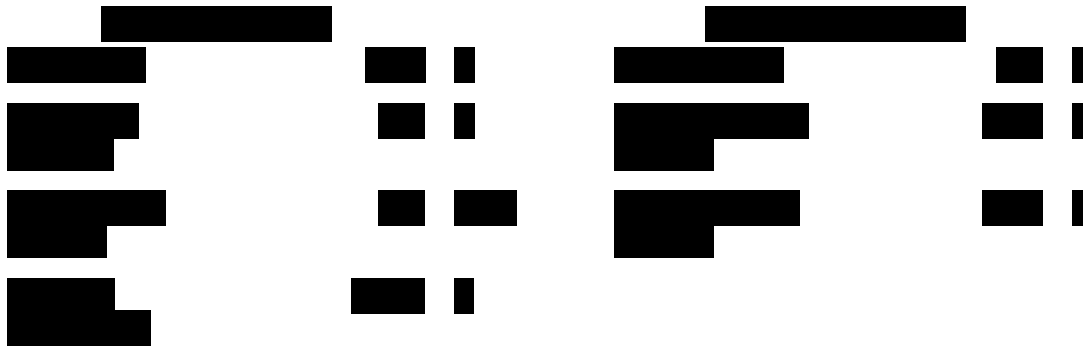
EXPOSED POPULATION: Adult

Number of Sites:

[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

[REDACTED]	[REDACTED]	[REDACTED] (Fugitive)	ASSUMPTIONS			
			ED (years)	AT (years)	BW (kg)	Inh. Rate (m³/hr)
Cancer						
LADD _{pot} (mg/kg/day)	1.48E-05	1.64E-04	30.00	75.00	71.80	0.55
LADC _{pot} (mg/m³)	8.04E-05	8.92E-04	30.00	75.00	NA	NA
Acute						
ADR _{pot} (mg/kg/day)	5.07E-04	1.41E-02	NA	1 day	71.80	0.55

Inhalation Comments:



Meteorological and Terrain Information:

Surrounding Land Use:	Rural
Terrain Height:	0.00 m
Distance to Residence of Interest:	100.00 m
Meteorological Class:	Full
Stability Class:	NA
Wind Speed:	NA

Downwash Information:

Facility Length:	NA m
Facility Width:	NA m
Facility Height:	NA m

LEGEND FOR NEW CHEMICALS EXPOSURE REPORT

This new chemicals exposure report was prepared by the Exposure Assessment Branch (EAB) of the Economics, Exposure and Technology Division, Office of Pollution Prevention and Toxics, USEPA.

The goals of these reports are to calculate conservative (protective) estimates of exposure endpoints for consumers, the general population, and the environment.

For each exposure scenario to industrial releases, the following three endpoints are calculated:

- (1) maximum possible acute concentrations and doses
- (2) maximum possible chronic concentrations and doses
- (3) for water releases ≥ 20 days, the probability of exceedence of the aquatic concentration of concern

These endpoints are identified by abbreviations on the Release Activity line, e.g., (1) **max ADR**, (2) **max LADD**, (3) **max PDM**. Depending on the release inputs, these endpoints may be calculated and presented on the same page or different pages. That is, a release activity ID of *mfg; max ADR, max PDM, max LADD* indicates that all the exposure endpoints were calculated from common manufacturing release values; conversely, a release activity ID of *mfg &proc; max ADR* indicates that only the maximum acute exposure values were calculated for manufacturing and processing releases that occurred at the same site.

For each consumer product use exposure scenario, whether exposure is to the user directly or to the general population/environment, the maximum exposure values are calculated and presented together.

In addition to the exposure values above, EPA policy directs that exposure and release values be compared to criteria threshold values for Exposure-based and PBT Exposure-based cases.

Exposure-based (YX) cases (those with $\geq 100,000$ kg/yr production volume)

Criteria are exceeded under the following conditions:

- Presence in consumer product with likely exposure
- $\geq 3\text{E-}3$ mg/kg/d exposure via air, fish ingestion or drinking water
- $\geq 10,000$ kg/yr release to environment (post-treatment)
- $\geq 1,000$ kg/yr release to water (post-treatment)

Persistent, Bioaccumulative, Toxic (PBT) chemicals of P2B2 rating or higher and production volume $\geq 20,000$ kg/yr

Criteria are exceeded as for YX cases, with the following differences:

- $\geq 2,000$ kg/yr release to environment (post-treatment)
- ≥ 200 kg/yr release to water (post-treatment)

Bolding rules in the Report: Values for endpoints above that are also health or eco concerns are bolded.